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- (71) Applicant (*for all designated States except US*): YALE UNIVERSITY [US/US]; 451 College Street, New Haven, CT 06520 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): ZHANG, Hui [US/US]; 203 Schoolside Lane, Guilford, CT 06437 (US). TSVETKOV, Lyuben, M. [BG/US]; 137 Cottage Street, Apt. E3, New Haven, CT 06517 (US). KONDO, Takeshi [JP/US]; 115 Florence Road, Apt. 1A, Branford, CT 06405 (US).
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(54) Title: MODULATION OF PROTEIN LEVELS USING THE SCF COMPLEX

(57) Abstract: This invention encompasses various methods of modulating protein levels using the SKP1, CDC53/Cullin, F-box(SCF) protein complex. More specifically, the present invention provides various methods of target protein degradation using targeted ubiquitination techniques. The present invention also provides various compositions and assays associated with the disclosed modulation of protein levels using the SCF complex as well as various methods of detecting, monitoring and treating cancerous cells.

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## MODULATION OF PROTEIN LEVELS USING THE SCF COMPLEX

### INVENTORS

Hui Zhang, Lyuben M. Tsvetkov and Takeshi Kondo

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### RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application No. 60/137,494 filed June 4, 1999 which is herein incorporated by reference in its entirety.

### 10 FIELD OF THE INVENTION

The present invention pertains, in general, to the field of protein knockout technology. In particular, the present invention pertains to protein knockout technology using targeted ubiquitination techniques.

### 15 BACKGROUND OF THE INVENTION

All publications, patents and patent applications discussed herein are incorporated by reference to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference. The paper by Tsvetkov *et al.*, (1999) Current Biology 9, 661-664 including S1-S2 is fully and completely herein incorporated by reference.

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#### Cyclin-Dependent Kinases (CDKs)

Entry into each phase of the eukaryotic cell division cycle is regulated by proteins known as cyclin-dependent kinases (CDKs). In mammalian cells, seven different CDK protein subtypes have been described, each of which has been associated with particular phases of the cell cycle (Matsushime *et al.*, (1992) Cell 71, 323-334; Xiong *et al.*, (1992) Cell 71, 505-514; Meyerson *et al.*, (1992) EMBO J. 11, 2909-2917; Fisher & Morgan, (1994) Cell 78, 713-724; Meyerson & Harlow, (1994) Mol. Cell. Biol. 14, 2077-2086). Activation of each CDK in the cell cycle is regulated by its association with an equally diverse family of regulatory subunits known as cyclins. Multiple cyclin-CDK associations have been implicated in cell cycle control during cell proliferation in mammals. For

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example, cyclin D-CDK4 is associated with cell cycle progression through G<sub>1</sub> phase while both cyclin E-CDK2 and cyclin A-CDK2 facilitate G<sub>1</sub> to S phase transition (Nasmyth & Hunt, (1993) Nature 366, 634-635).

CDKs are regulated at several different levels including phosphorylation and  
5 interaction with other proteins. Activation of CDKs is initially dependent on complex formation with their cognate cyclin subunits and is regulated at this stage by fluctuations in the levels of these subunits (Sherr, (1993) Cell 73, 1059-1065). Phosphorylation of a conserved threonine residue in CDK is essential for activation following cyclin-CDK complex formation (Solomon *et al.*, (1993) EMBO J. 12, 3133-3142; Makela *et al.*, (1994)  
10 Nature 371, 254-257). Studies have also focused on the role of CDK inhibitor proteins such as p16, p21 or p27, which act as another level of cell cycle regulation by preventing unscheduled entry into another phase of the cell cycle (Hunter & Pines, (1994) Cell 79, 573-583; Sherr, (1996) Science 274, 1672-1677). These proteins interact with specific domains surrounding the phosphorylated threonine residue on the CDK. p27 for example,  
15 inhibits cyclin E-CDK2 and has been characterized in detail (Polyak *et al.*, (1994) Cell 78 59-36; U.S. Patent No. 5,688,665).

Transformed cells differ from normal cells in their ability to proliferate indicating that alterations in pathways which control cell cycle progression accompany cellular transformation. Alterations in the regulatory events underlying cellular proliferation  
20 pathways can translate into changes in the cyclin-CDK pathways controlling cell cycle progression and has long been implicated in cellular transformation. In normal cells each cyclin-CDK complex exists in a quaternary complex that also contains proliferating cell nuclear antigen (PCNA) and a CDK inhibitor protein. These quaternary complexes are absent in transformed cells because the CDK inhibitory protein is not expressed (Zhang *et al.*, (1993) Mol. Biol. Cell 4, 897-906).  
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For example, studies in normal human fibroblasts demonstrated that cyclin A-CDK2 was associated with p21 and PCNA in a quaternary complex. p21 and PCNA were absent in other transformed cells or established tumor cell lines, and cyclin A-CDK2 was bound to three novel proteins to form a protein complex (Zhang *et al.*, (1993) Mol.  
30 Biol. Cell 4, 897-906). The first two proteins in this complex were S-phase kinase associated proteins designated SKP1 and SKP2. The third protein, designated CUL1, is a

member of the cullin/CDC53 family of proteins. The cyclin A-CDK2/SKP1/SKP2/CUL1 complex functions as a conserved ubiquitin E3 enzyme that regulates mammalian G<sub>1</sub> to S phase transition by specifically targeting mammalian G<sub>1</sub> regulators, such as p21 for ubiquitin-dependent degradation (Yu *et al.*, (1998) Proc. Natl. Acad. Sci. USA 95, 11324-11329). Decreased levels of p21 in tumor cells confirm that p21 is being targeted for ubiquitin-dependent degradation in transformed cells (Xiong *et al.*, (1993) Genes Dev. 7, 1572-1583; Yu *et al.*, (1998) Proc. Natl Acad. Sci. USA 95, 11324-11329).

#### Ubiquitin-Dependent Protein Degradation

Ubiquitin-dependent protein degradation functions to regulate protein turnover in a cell by closely regulating the degradation of specific proteins. Once a protein is tagged with ubiquitin it is degraded in an ATP-dependent reaction by the 26S proteasome. Ubiquitin is a small protein composed of seventy-six amino acids that serves only as a tag to mark proteins for degradation. Three distinct enzymes are required for protein ubiquitination (King *et al.*, (1996) Science 274, 1652-1659). First, ubiquitin is activated in an ATP dependent reaction by forming a thioester bond with the ubiquitin activation enzyme designated E1. The activated ubiquitin is then transferred from E1 to the ubiquitin conjugating enzyme designated E2. This enzyme mediates the transfer of ubiquitin to protein substrates in conjunction with a ligase enzyme designated E3. The ubiquitinated protein substrates are then degraded by the 26S proteasome.

#### S-Phase Kinase Associated Proteins

In many DNA viral oncoprotein transformed or other established tumor cells that are deficient in p53 expression, p21 and proliferating cell nuclear antigen (PCNA) disappeared and cyclin A/CDK2 was prominently complexed with two novel proteins, S-phase kinase associated proteins 1 and 2 (SKP1 and SKP2, also known as p19 and p45, respectively) (Yu *et al.*, (1998) Proc. Natl. Acad. Sci. USA 95, 11324-11329; Zhang *et al.*, (1997) WO9711176). SKP1 and SKP2 have been isolated and the genes encoding these proteins have been sequenced (Zhang *et al.*, (1997) WO9711176; which is herein incorporated by reference in its entirety). SKP2 expression has been shown to be highly



induced in many transformed cells (Zhang *et al.*, (1995) Cell 82, 915-925, which is herein incorporated by reference in its entirety).

The SKP1/SKP2/CUL1 E3 ligase complex has been implicated in the ubiquitin-dependent degradation of p21 during cell cycle progression. Furthermore, p27 has also been shown to be a target of ubiquitin-dependent degradation in a CDC34-dependent proteolytic process. CDC34 serves as a ubiquitin E2 conjugating enzyme for SCF (SKP1, CDC53/Cullin, F-box protein) complexes (Yu *et al.*, (1998) Proc. Natl. Acad. Sci. USA 95, 11324-11329; Pagano *et al.*, (1995) Science 269, 682-685; King *et al.*, (1996) Science 274, 1652-1659). The ubiquitin-dependent p27 degradation occurs during the transition from G1 to S phase as indicated by the increase in the level of SKP2 in late G1 which corresponds with a decrease in p27 levels. p27 ubiquitin-dependent degradation is also dependent on cyclin E/CDK2 activity (Brandeis & Hunt, (1996) EMBO J. 15, 5280-5289; Sheaff *et al.*, (1997) Genes Dev. 11, 1464-1478).

## SUMMARY OF THE INVENTION

The present invention encompasses a method of altering the level of polypeptide in a cell comprising altering the amount of one or more of the proteins selected from the group consisting of SKP1, SKP2, SKP2-like protein and CUL-1. In a preferred embodiment, the polypeptide is phosphorylated and the SKP2-like protein is selected from the group consisting of ZF1 (SEQ ID NO: 27), ZF3 (SEQ ID NO: 29), ZF4 (SEQ ID NO: 31), ZF5 (SEQ ID NO: 33), ZF6 (SEQ ID NO: 35), ZF7 (SEQ ID NO: 37), ZF8 (SEQ ID NO: 39), ZF9 (SEQ ID NO: 41), ZF11 (SEQ ID NO: 43), ZF13 (SEQ ID NO: 45), ZF16 (SEQ ID NO: 47), ZF18 (SEQ ID NO: 49), ZF19 (SEQ ID NO: 51), ZF20 (SEQ ID NO: 53), ZF23 (SEQ ID NO: 55), ZF24 (SEQ ID NO: 57), ZF25 (SEQ ID NO: 59) and ZF26 (SEQ ID NO: 61).

In yet another preferred embodiment the polypeptide in the method of the invention is selected from the group consisting of p27 (SEQ ID NO: 65), cyclin E (SEQ ID NO: 63), Max (SEQ ID NO: 9), Mad (SEQ ID NO: 11), c-Myc (SEQ ID NO: 13), MDM2 (SEQ ID NO: 15), p53 (SEQ ID NO: 17), Bax (SEQ ID NO: 19), Bad (SEQ ID NO: 21) and Bcl-2 (SEQ ID NO: 23). The method of invention may be used to increase

the level of polypeptide by decreasing the amount of SKP2 or in the alternative the level of polypeptide is reduced by increasing the amount of SKP2.

In a yet another embodiment, the invention includes a method of altering the level of SKP2 comprising altering the amount of p27 polypeptide which is available for binding with SKP2. In a further embodiment, the invention includes a method of modulating the activity of SKP2 comprising contacting SKP2 with a peptide comprising a SKP2 interaction domain which is available for binding with SKP2. In a preferred embodiment, the peptide is phosphorylated and the SKP2 interaction domain is derived from p27 or cyclin E. In a preferred embodiment, the peptide comprises any one of the amino acid sequences of SEQ ID NO: 1, 2, 3, 4, 5 or 6.

The invention also includes a method of treating a tumor in a mammal comprising altering the level of SKP protein in the cells of said tumor. In a preferred embodiment the SKP protein is SKP2 or allelic variants thereof. In a related embodiment the invention includes a method of detecting a tumor in a mammal wherein the level of SKP2 is used as a diagnostic and prognostic indicator to determine the progression of said tumor. In a preferred embodiment, the invention encompasses a method of monitoring the treatment of a tumor in a mammal wherein the level of SKP2 is used as a diagnostic and prognostic indicator.

The invention also includes methods of testing an agent for the ability to modulate an interaction between SKP2 and a target protein wherein the method comprises (a) fusing SKP2 with a target protein interaction domain to produce a SKP2 fusion protein; (b) contacting the agent, the SKP2 fusion protein and the target protein; and (c) determining whether the interaction of the SKP2 fusion protein with the target protein has been modulated by the agent.

The invention further encompasses a method of altering the level of a target protein in a cell comprising inserting a heterologous target protein interaction domain with SKP2 or a SKP2-like protein to produce a fusion protein, and contacting fusion protein with the target protein. In a preferred embodiment, the SKP-2 like protein is selected from the group consisting of ZF1 (SEQ ID NO: 27), ZF3 (SEQ ID NO: 29), ZF4 (SEQ ID NO: 31), ZF5 (SEQ ID NO: 33), ZF6 (SEQ ID NO: 35), ZF7 (SEQ ID NO: 37), ZF8 (SEQ ID NO: 39), ZF9 (SEQ ID NO: 41), ZF11 (SEQ ID NO: 43), ZF13 (SEQ ID NO: 45), ZF16 (SEQ

ID NO: 47), ZF18 (SEQ ID NO: 49), ZF19 (SEQ ID NO: 51), ZF20 (SEQ ID NO: 53), ZF23 (SEQ ID NO: 55), ZF24 (SEQ ID NO: 57), ZF25 (SEQ ID NO: 59) and ZF26 (SEQ ID NO: 61).

5 In yet another embodiment, the invention includes a method of altering the level of a target protein in a cell comprising expressing a cDNA coding for a SKP2 fusion protein comprising a SKP2 protein fused with a target protein interaction domain which is specific for the target protein. In a related embodiment, the invention includes a method of ubiquitinating a target protein comprising fusing a target protein interaction domain with SKP2, and contacting the SKP2 fusion protein with the target protein. In preferred  
10 embodiments, the target protein is selected from the group consisting of p27 (SEQ ID NO: 65), cyclin E (SEQ ID NO: 63), Max (SEQ ID NO: 9), Mad (SEQ ID NO: 11), c-Myc (SEQ ID NO: 13), MDM2 (SEQ ID NO: 15), p53 (SEQ ID NO: 17), Bax (SEQ ID NO: 19), Bad (SEQ ID NO: 21) and Bcl-2 (SEQ ID NO: 23).

The invention also includes a method of modulating protein ubiquitination  
15 comprising altering the amount of SKP2 which is available to facilitate protein ubiquitination.

Finally, the invention encompasses a fusion protein comprising a first protein comprising at least one SKP2 C-terminal motif (SCM) capable of interacting with SKP1 and forming a complex with CUL-1 and a second protein which is capable of interacting  
20 with a heterologous target protein. In a preferred embodiment, the fusion protein contains only one SCM capable of interacting with SKP1. In another preferred embodiment, the SCM is selected from any one of the following proteins selected from the group consisting of SKP2 (SEQ ID NO: 67), ZF1 (SEQ ID NO: 27), ZF3 (SEQ ID NO: 29), ZF4 (SEQ ID NO: 31), ZF5 (SEQ ID NO: 33), ZF6 (SEQ ID NO: 35), ZF7 (SEQ ID NO: 37), ZF8 (SEQ  
25 ID NO: 39), ZF9 (SEQ ID NO: 41), ZF11 (SEQ ID NO: 43), ZF13 (SEQ ID NO: 45), ZF16 (SEQ ID NO: 47), ZF18 (SEQ ID NO: 49), ZF19 (SEQ ID NO: 51), ZF20 (SEQ ID NO: 53), ZF23 (SEQ ID NO: 55), ZF24 (SEQ ID NO: 57), ZF25 (SEQ ID NO: 59) and ZF26 (SEQ ID NO: 61).

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## BRIEF DESCRIPTION OF THE DRAWINGS

### Figure 1 - Phosphorylation-dependent p27 degradation in HeLa extracts

(A) *In vitro* translated,  $^{35}\text{S}$ -labeled p27 or p27 T187G mutant was incubated with HeLa extracts for 3 hours at 30 °C. The addition of cyclin E/CDK2 and the 26S proteasome inhibitor, MG132 (20  $\mu\text{l}$ ) is indicated. The p27 reaction products were isolated by immunoprecipitation and visualized by autoradiography. (B) Time course of p27 degradation using baculovirus produced and  $^{35}\text{S}$ -labeled p27 (0.5  $\mu\text{g}$ ). (C) Baculovirus-expressed,  $^{35}\text{S}$ -labeled p27 was incubated with HeLa extracts in the absence or presence of cyclin E/CDK2 and MG132. The reaction products were treated with lambda phosphatase (PPTase). The phosphorylated and high molecular weight p27 species (in brackets) are indicated.

### Figure 2 - Ubiquitination of p27 in the HeLa cytosolic extracts

Accumulation of ubiquitinated p27 in the presence of modified ubiquitins. p27 was incubated with HeLa extracts, cyclin E/CDK2, and methyl ubiquitin (UbM, 0.5 mg/ml) and ubiquitin aldehyde (UbA, 1  $\mu\text{M}$ ) as indicated. The ubiquitinated p27 ladders accumulated because methylated ubiquitin shortens the polyubiquitinated chain and thus slows down the rate of degradation while ubiquitin aldehyde inhibits de-ubiquitination of ubiquitinated proteins by isopeptidases.

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### Figure 3 - Inhibition of p27 degradation by depletion of the SCF<sup>SKP2</sup> complex

(A) Depletion of CUL-1 abolishes p27 degradation. HeLa extracts were passed through the affinity purified CUL-1 antibody or a control IgG column. The control and CUL-1 depleted extracts were assayed for p27 degradation activity at various times at 30 °C in the presence of cyclin E/CDK2. The left three lanes are p27 input and regular HeLa extracts and incubated for three hours. Reaction products were treated with lambda phosphatase. (B) SKP1 depleted extracts. The extracts were control depleted using IgG or depleted with an SKP1 antibody column and then incubated with p27 as described in A. The reaction products were not treated with the phosphatase so the phosphorylated p27 are shown. Ext: regular HeLa extracts. (C) SKP2 depleted extracts. SKP2 was immuno-depleted as described in A and B. The control and SKP2 depleted extracts were incubated for three

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hours at 30 °C and the reaction products were analyzed after phosphatase treatment. (D) Specific removal of SKP2, SKP1, and CUL-1 by the immuno-depletion processes. The regular HeLa (Ext), control depleted, and SKP2, SKP1 or CUL-1 depleted extracts were Western-blotted by SKP2 (top), SKP1 (middle), and CUL-1 (bottom) antibodies, respectively.

Figure 4 - SCF<sup>SKP2</sup> complex specific interactions with thr187 phosphorylated p27 peptide

(A) Sequences of p27 carboxy-terminal peptides (amino acids 175-198) with or without threonine 187 phosphorylation. (B) SKP2 specifically binds to the threonine 187

phosphopeptide of p27. F-box proteins, SKP2,  $\beta$ -TrCP, and MD6 were *in vitro* translated as <sup>35</sup>S-labeled proteins. The proteins (10  $\mu$ l each) were incubated for one hour with the p27 peptide or threonine 187 phosphopeptide beads. The proteins associated with the beads were purified and analyzed. (C) Selective binding of SKP2, SKP1 and CUL-1 in the HeLa extracts to the p27 threonine 187 phosphopeptide. HeLa extracts (400  $\mu$ g) were incubated with the p27 phosphopeptide or peptide beads for one hour. The proteins associated with the beads were analyzed by Western-blot analysis with either SKP2, SKP1, CUL-1 or  $\beta$ -TrCP antibodies, as indicated. The immunoprecipitated SKP2, SKP1 and CUL-1 are included as a control as indicated. For lane four,  $\beta$ -TrCP, HeLa extracts (25  $\mu$ g) were directly loaded without immunoprecipitation. HeLa extracts for SKP2 immunoprecipitation were 25  $\mu$ g while for SKP1 and CUL-1 were 100  $\mu$ g. (D)

Association between SKP1, SKP2 and CUL-1 in the HeLa extracts. The SKP1, SKP2 and CUL-1 were immunoprecipitated by specific antibodies, respectively, as indicated. The immunoprecipitated proteins were examined for the presence of SKP2 by Western-blotting with SKP2 antibodies. (E) Association between SKP1 and p27 phosphopeptide depends on the presence of SKP2. HeLa (Ext), Mock or SKP2 depleted extracts (100  $\mu$ g each) were incubated either with p27 peptide (pept) or phosphopeptide (phosphopept) beads. The proteins associated with the peptide beads were examined for the presence of SKP2 or SKP1 by Western blot. SKP2 and SKP1 in the mock and SKP2 depleted extracts (25  $\mu$ g each) were also examined by direct Western blotting of the extracts. Depl: depletion of extracts by pre-immune IgG (Mock) or SKP2 antibodies.

Figure 5 - SCF<sup>SKP2</sup> complex contains a p27 ubiquitination E3 activity

(A) Restoration of p27 degradation activity in SKP2 depleted extracts by recombinant SCF<sup>SKP2</sup>. Insect SF9 cells were co-infected with baculoviruses encoding GST-SKP1 and CUL-1, either in the presence (SCF<sup>SKP2</sup>) or in the absence of SKP2 (SC) baculoviruses.

5 The SCF<sup>SKP2</sup> and SC complexes were isolated by glutathione Sepharose. The recombinant SCF<sup>SKP2</sup>, SC (one µg each in two µl), or the buffer (two µl) was added into the SKP2 depleted extracts (200 µg), as indicated, and p27 degradation was assayed in the presence of cyclin E/CDK2. HeLa extract (ext) was included as the control. (B) p27 ubiquitination using recombinant proteins. p27 was incubated with the recombinant SCF<sup>SKP2</sup> complex, in  
10 the presence of purified cyclin E and cyclin A/CDK2 kinases, human E1 ubiquitin activating enzyme, ATP, ubiquitin, and recombinant CDC34, an E2 ubiquitin conjugation enzyme (lane 2). The reactions in lanes 3 and 4 were conducted in the absence of either ubiquitin or CDC34, respectively.

15 Figure 6 - SKP2 specifically binds to the phosphorylated Thr380 in cyclin E

(A) Sequences of the cyclin E carboxy-terminal peptides (residues 371-394) with (TP-CP) or without Thr380 (TP-C) phosphorylation. (B) SKP2 is specific for cyclin E

phosphopeptide binding. *In vitro*-translated and <sup>35</sup>S-labeled F-box proteins SKP2, FBL2, 5, 6, 7, and 8 (10 µl each) were incubated with the TP-C or TP-CP beads. Their

20 associations with cyclin E peptide beads were analyzed. Lysates: translated lysate control.

(C) Specific interaction between SKP2 and the Thr380-phosphorylated cyclin E peptide in HeLa cell extracts. 400 µg of HeLa cytosolic extracts were incubated with either TP-C or TP-CP beads (25 µl) for one hour. The proteins associated with the beads were Western-blotted with antibodies against SKP2, SKP1, or CUL-1. Left lane, HeLa lysate control.

25 (D) Upper panel - The phosphorylated TP motif is required for specific SKP2 interaction. Mutant derivatives of cyclin E peptides were synthesized in which either Thr380 is converted into serine or phosphoserine (SP-C or SP-CP) or Pro381 is converted into alanine in TP-CP (TA-CP). The beads bearing the wild-type and the mutant cyclin E peptides were assayed for SKP2 binding as described in (C). Lower panel - competition of  
30 the interaction between SKP2 and cyclin E Thr380 peptide beads (TP-CP) with wild-type or mutant cyclin E phosphopeptides. 25 µl TP-CP beads were incubated with HeLa

extracts (400 µg) in the absence or in the presence of either TP-CP (10, 50 or 250 µg/ml) or equal amounts of TA-CP or SP-CP. The association between SKP2 and the TP-CP beads was analyzed by Western-blot.

5     Figure 7 - SKP2 promotes cyclin E ubiquitination and degradation

- (A) Dependency of SKP2-mediated cyclin E degradation on Thr380 in cyclin E. T7-cyclin E or cyclin E T380G mutant constructs were transfected into HeLa cells in the presence or absence of SKP2 expression vector. Cell lysates were prepared in an SDS-containing buffer and 40 µg of each lysate were loaded directly onto a protein gel. The
- 10    proteins were detected by Western-blotting with anti-T7 (top), SKP2 (middle), and CDK2 (lower) antibodies. (B) SKP2-induced formation of high molecular-weight species of cyclin E is sensitive to the Thr380 mutation in cyclin E. One microgram of T7-cyclin E or cyclin E T380G mutant expression constructs was transfected into 293 cells in the absence or presence of increasing amounts of the SKP2 construct (0, 0.25, 0.5, 1 and 2.5 µg,
- 15    respectively). The proteins were detected by anti-T7 (top) or SKP2 (lower) antibodies. (C) SKP2 and ubiquitin both induce high-molecular-weight species of cyclin E. Expression vectors encoding SKP2 (5 µg), T7-tagged cyclin E (1 µg), or HA-tagged ubiquitin (HAUb, 1 µg) were transfected into 293 cells as indicated. Twenty-four hours post-transfection, cells were treated with LLNL for six hours. The proteins were detected
- 20    by either anti-T7 monoclonal (top and middle panels) or anti-SKP2 (lower panel) antibodies. The middle panel is a lighter exposure of the top panel. (D) SKP2 promotes polyubiquitination of cyclin E. Expression vectors encoding SKP2 (5 µg), T7-tagged cyclin E (1 µg), or HA-tagged ubiquitin (HAUb, 0.1 µg) or a combination of them were transfected into 293 cells as indicated. The proteins were immunoprecipitated with the
- 25    anti-HA antibody (12CA5) for ubiquitinated proteins followed by Western-blotting with anti-T7 antibody for cyclin E. (E) SKP2-mediated cyclin E ubiquitination is p27-independent but requires Thr380. p27<sup>-/-</sup> mouse embryonic fibroblasts were transfected with T7-cyclin E, SKP2 expression constructs, or both as described in B. The proteins were detected by anti-T7 (top) or SKP2 (lower) antibodies.

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Figure 8 - SKP2 affects cyclin E stability by directly binding to cyclin E

(A) SKP2 shortens the half-life of the cyclin E protein. Tagged-cyclin E expression construct was transfected into HeLa cells in the absence or in the presence of SKP2. Twenty-four hours after transfection, the cells were pulse-labeled with <sup>35</sup>S-methionine for thirty minutes. The labeling medium was removed and the cells were chased in fresh medium containing 1 mM unlabeled methionine. The cells were harvested at various points (0, 1, 2, 3 and 4 hours) in the chasing medium and the labeled cyclin E protein was immunoprecipitated and examined. (B) Association of cyclin E with SKP2 *in vivo*. p27<sup>-/-</sup> mouse embryonic fibroblasts were transfected with DNA expression constructs encoding LacZ (β-Gal), T7-cyclin E, or the T380G cyclin E mutant. The lysates were prepared and immunoprecipitated with anti-cyclin E (left) or anti-SKP2 antibodies. The presence of cyclin E in the immunoprecipitates was examined with the anti-T7 antibody by Western-blotting. (C) Cyclin E degradation is inhibited by p27. SKP2 (5 μg), T7-tagged cyclin E (1 μg), LacZ (β-Gal), or p27 T187G mutant (1 μg) expression constructs were transfected into HeLa cells as indicated. The levels of cyclin E and p27 T187G mutant were detected with T7 and p27 antibodies.

#### Figure 9 - Effects of SKP2 on endogenous cyclin E

(A) SKP2 decreases the levels of endogenous cyclin E. U87EcoR cells were infected with recombinant retroviruses encoding either LacZ (β-gal) or SKP2. Thirty-six or sixty hours after infection, cell lysates were prepared and 40 μg of lysates were used for examination of the levels of endogenous cyclin E, CDK2, p27 and the expression of SKP2 by Western-blotting using their specific antibodies. (B) SKP2 induces cyclin E down-regulation in S-phase cells. Thirty-six hours post-retrovirus-infection, cells were treated with 5 mM HU for twenty-four more hours to synchronize cells in S phase. The levels of endogenous cyclin E and CDK2 as well as the expression of SKP2 were examined. (C) Expression of a dominant negative SKP2 mutant causes the accumulation of endogenous cyclin E. Glioblastoma U87EcoR cells were infected with recombinant retroviruses containing either an empty vector or a SKP2 dominant negative mutant (SKP2DN). The levels of either endogenous cyclin E, p27, SKP2 as well as the exogenous SKP2DN were examined forty-eight hours after infection. (D) The dominant negative effect of the SKP2 mutant on



cyclin E accumulation is p27-independent. The experiment was performed in essentially the same way as in C, except that p27<sup>-/-</sup> mouse embryonic fibroblasts were used.

Figure 10 - Alteration of the substrate-specificity of F-box proteins

- 5 The  $\beta$ -TRCP and SKP2 hybrid protein was generated to alter the substrate-specificity of  $\beta$ -TRCP to that of SKP2. The cDNA encoding the amino-terminus domain of  $\beta$ -TRCP (residues 1-204, including the F-box) was amplified with PCR and cloned into Bluescript at XhoI site. The cDNA containing the carboxy-terminus region of SKP2 without the F-box but retaining the LRR region (residues 169-435, without the F-box) was similarly
- 10 amplified and fused with the amino-terminal region of  $\beta$ -TRCP. The resulting cDNA encoding the TRCP.N/SKP2.C hybrid protein is cloned into pcDNA3 under CMV promoter control. The corresponding truncated SKP2 carboxy-terminal region (SKP2.C) or the amino-terminal region of  $\beta$ -TRCP ( $\beta$ -TRCP.N) was also cloned into pcDNA3.

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Figure 11 - TRCP.N/SKP2.C hybrid induces formation of polyubiquitinated cyclin E

- One microgram of T7-tagged cyclin E were transfected into 293 human embryonic kidney cells in the presence of either the control empty vector, SKP2, SKP2 amino-terminal region (SKP2.N, residues 1-168),  $\beta$ -TRCP amino-terminal region (TRCP.N),
- 20 TRCP.N/SKP2.C hybrid, or SKP2 carboxy-terminal region (5  $\mu$ g each) by the calcium phosphate method. Both SKP2.N and TRCP.N contain the F-box. Cell lysates were prepared twenty-four hours post-transfection in an SDS-containing buffer and 40  $\mu$ g of each lysate were loaded directly onto a protein SDS-PAGE gel. The proteins were detected by Western-blotting with anti-T7 antibodies for the transfected cyclin E.

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Figure 12 - F-box amino acid sequence alignment

Homologies within the F-box region between various F-box containing proteins.

Figure 13. - SCM domain amino acid sequence alignment

- 30 Homology between the  $\alpha$  domain of the von Hippel-Linda protein (VHL) (SEQ ID NO: 73) and the SCM domain of SKP2.

Figure 14 - Dependency of SKP2-mediated cyclin E degradation on Thr380 in cyclin E

T7-cyclin E or cyclin E T380G mutant constructs were transfected into HeLa cells in the presence or absence of SKP2 expression vector. Cell lysates were prepared and 40 µg of  
5 each lysate was loaded directly onto a protein SDS PAGE gel. The proteins were detected by Western-blotting with anti-T7 (upper) and SKP2 (lower) antibodies.

Figure 15 - Isolated SCF<sup>SKP2</sup> complex contains ubiquitination activity

The immunoprecipitated complex was incubated for 1 hour at 30°C with 6 µM ubiquitin, 2  
10 mM ATP, 50 mM creatine phosphate, 20 µg/ml creatine kinase, 1 µg purified ubiquitin activating enzyme E1, 1 µg purified E2 conjugating enzyme CDC34 in a buffer containing 20 mM Hepes, pH 7.2, 10 mM MgCl<sub>2</sub>, 1 mM DTT. The ubiquitin reaction was terminated by addition of 0.5% SDS and loaded directly in an SDS-PAGE protein gel. The ubiquitinated proteins were detected by Western-blotting with the anti-ubiquitin antibody  
15 (Chemcon International).

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this  
20 invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described.

### A. Definitions

25 As used herein, the term "agent" means any molecule that is randomly selected or rationally designed. As used herein, an agent is said to be randomly selected when the agent is chosen randomly without considering the specific sequences involved in the association of the proteins under study or the known functions of the proteins under study. An example of randomly selected agents is the use a chemical library, a peptide  
30 combinatorial library, or a growth broth of an organism.

As used herein, an agent is said to be “rationally designed” when the agent is chosen on a non-random basis which takes into account the sequence of the proteins under study and/or their conformation in connection with the agent’s action. Agents can be rationally selected or rationally designed by utilizing the amino acid sequences that make up potential contact sites between the proteins. For example, a rationally selected peptide agent can be a peptide whose amino acid sequence is identical to an identified contact site on one of the proteins under study. Such an agent will reduce or block the association of the protein with its binding partner by binding to the contact site on the first protein.

The agents of the present invention can be, as examples, peptides, small molecules, nucleic acids, vitamin derivatives, as well as carbohydrates. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents of the present invention.

The peptide agents of the invention can be prepared using standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the DNA encoding these peptides may be synthesized using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production using solid phase peptide synthesis is necessitated if non-gene-encoded amino acids are to be included.

Another class of agents are antibodies immunoreactive with one of the proteins under study. Particularly useful are antibodies immunoreactive with the extracellular domain of membrane proteins under study. As described above, antibodies are obtained by immunization of suitable mammalian subjects with peptides, containing as antigenic regions, those portions of the protein intended to be targeted by the antibodies. Critical regions include the contact sites between the two proteins as well as extracellular regions of membrane proteins.

As used herein, the term “agonist” includes those agents, compounds, compositions, etc. which when administered can up-regulate (increase, promote or otherwise elevate the level of) a particular protein.

As used herein, an “allelic variant” refers to a proteins having different amino acid sequences than those sequences listed herein or incorporated by reference. For example, the allelic variants of p27, the target protein interaction domain of p27, SKP1, SKP2 or

SKP2-like proteins, or CUL-1, though possessing a slightly different amino acid sequence, such as a conservative amino acid substitution, than those disclosed herein or incorporated by reference, will still have the requisite biological activity of the native protein. As used herein, a “conservative amino acid substitution” refers to alterations in the amino acid  
5 sequence of a protein which do not adversely effect their native abilities. Allelic variants, conservative substitution variants and related proteins and protein fragments utilized herein preferably will have an amino acid sequence having at least about 75% amino acid sequence identity with the published sequences, more preferably at least about 80%, even more preferably at least about 90%, and most preferably at least about 95%.

10 Thus, the peptides, variants and related molecules that are the subject of or utilized in this invention include molecules having the sequences disclosed; fragments thereof having a consecutive sequence of at least about 3, 5, 10, 15, 20, 25, 30, 50 or more amino acid residues from the corresponding native proteins and amino acid sequence variants of such proteins, or their fragments as defined above, that have been conservatively  
15 substituted by another residues.

As used herein, the term “altering the level” of a particular protein means either increasing or decreasing the amount of that protein. For example, “altering the level of SKP2” means either increasing or decreasing the amount of SKP2.

As used herein, the term “antagonist” includes those agents, compounds,  
20 compositions, etc. which when administered cause the down regulation (inhibition, prevention, reduction, etc.) of a particular protein.

As used herein, the term “fusion protein” means a hybrid protein including a synthetic or heterologous amino acid sequence. A fusion protein can be produced, for example, from a hybrid gene containing operatively linking heterologous gene sequences.

25 As used herein, the terms “isolated DNA, RNA, peptides, polypeptides, or proteins” means DNA, RNA, peptides, polypeptides or proteins that are isolated or purified relative to other DNA, RNA, peptides, polypeptides or proteins in the source material. For example, “isolated DNA” that encodes SKP2 (which would include cDNA) refers to DNA purified relative to DNA which encodes polypeptides other than SKP2.

30 As used herein, the term “modulating the activity” of a particular protein means affecting the covalent or noncovalent binding of that protein with another protein. For

example, when referring to “modulating the activity” of SKP2 this means affecting the binding of SKP2 with another protein, such as p27 or a peptide which includes the SKP2 interaction domain of p27.

As used herein, the term “pharmaceutically acceptable” refers to molecular entities  
5 and compositions such as fillers and excipients that are physiologically tolerated and do not typically produce an allergic or toxic reaction, such as gastric upset, dizziness and the like when administered to a subject or a patient; the preferred subjects of the invention are vertebrates, mammals and humans.

As used herein, the term “polypeptide” refers to a peptide which on hydrolysis  
10 yields more than two amino acids, called tripeptides, tetrapeptides, etc. according to the number of amino acids contained in the polypeptide. The term “polypeptide” is used synonymously with the term “protein” and “peptide” throughout the specification.

As used herein, “SCF” refers to a triple protein ligase consisting of SKP, Cullin and E-Box.

15 As used herein, “SCM” refers to a SKP2 C-terminal motif.

As used herein, “SKP” refers to a S-phase kinase associated protein. Specific examples of SKP proteins include, but are not limited to, SKP1, SKP2 and SKP2-like proteins.

As used herein, “SKP2-like protein” refers to a protein which can replace SKP2 to  
20 form a complex with SKP1 and CUL-1 or their yeast homologs. SKP2-like proteins are proteins that contain a SKP1 interacting domain that is homologous to the SKP1 interacting domain of the SKP2 sequence. Specific examples of SKP2-like proteins include, but are not limited to, ZF1 (SEQ ID NO: 27), ZF3 (SEQ ID NO: 29), ZF4 (SEQ ID NO: 31), ZF5 (SEQ ID NO: 33), ZF6 (SEQ ID NO: 35), ZF7 (SEQ ID NO: 37), ZF8  
25 (SEQ ID NO: 39), ZF9 (SEQ ID NO: 41), ZF11 ( $\beta$ -TRCP) (SEQ ID NO: 43), ZF13 (SEQ ID NO: 45), ZF16 (SEQ ID NO: 47), ZF18 (SEQ ID NO: 49), ZF19 (SEQ ID NO: 51), ZF20 (SEQ ID NO: 53), ZF23 (SEQ ID NO: 55), ZF24 (SEQ ID NO: 57), ZF25 (SEQ ID NO: 59) and ZF26 (SEQ ID NO: 61).

As used herein the term “SKP1 interacting domain” refers to the region on the  
30 SKP2 protein that interacts with the SKP1 protein. This region is also called the F-box for SKP1 binding.

As used herein the term “SKP2 interacting domain” refers to the region on a protein other than SKP2 that interacts with the SKP2 protein.

As used herein, the term “target protein” refers to an autologous or heterologous protein other than SKP2 which is targeted for interacting with a SKP2 or a SKP2-like protein.

As used herein, the term “target protein interaction domain” refers to a sequence which when fused to SKP2 or a SKP2-like protein interacts with a target protein.

As used herein, the term “ubiquitin” refers to a polypeptide found in all eukaryotic cells that participates in a variety of cellular functions including protein degradation.

As used herein, the terms “ubiquitinating” and “ubiquitination” refer to processes whereby ubiquitin is attached to a protein.

#### B. SKP2-mediated Degradation of Target Proteins

Applicants have identified SKP2 as an F-box protein that mediates ubiquitin-dependent degradation of p27 (SEQ ID NO: 65) and cyclin E (SEQ ID NO: 63). SKP2 (SEQ ID NO: 67) is an F-box protein that is expressed in late G1, S, and G2 phases, playing a role in S phase of the cell cycle (Zhang *et al.*, (1995) Cell 82, 915-925). SCF<sup>SKP2</sup> binds and targets the CDK inhibitor p27 for ubiquitin-dependent degradation. In addition, SKP2 also interacts with cyclin E and plays a role in the ubiquitin-dependent degradation of cyclin E. The present invention therefore includes methods for SKP2-mediated degradation of autologous and heterologous proteins. This SKP2-mediated cyclin E ubiquitination and degradation is mostly dependent on the presence of Thr380 in cyclin E, although weak cyclin E ubiquitination in the absence of Thr380 was also promoted by SKP2 *in vivo*.

Although cyclin E ubiquitination is independent of p27, in the presence of co-expressed CDK inhibitor p27, cyclin E degradation was inhibited even in the presence of SKP2 (Figure 8C). This observation indicates that p27 might inhibit cyclin E autophosphorylation on Thr380, leading to resistance to SKP2-mediated ubiquitin-dependent degradation of cyclin E. The effect of p27 is not to be due to a competition between p27 and cyclin E for SKP2 binding, since a non-phosphorylated mutant form of p27 in which the critical Thr187 was converted into glycine (T187G) cannot bind to

SKP2. This data is consistent with the previous report that p27 inhibits the Thr380-dependent cyclin E degradation (Clurman *et al.*, (1996) Genes Devel. 10, 1979-1990) and indicates that SKP2-mediated cyclin E ubiquitination is p27-independent.

Applicants have also identified that SKP2 performs a dual function during the G1/S transition. It is required for the ubiquitin-dependent degradation of p27 in late G1. The degradation of p27 by SCF<sup>SKP2</sup> activates cyclin E/CDK2 and promotes entry into the S-phase (Sutterluty *et al.*, (1999) Nat. Cell. Biol. 1, 207-14; Coats *et al.*, (1996) Science 272, 877-880). Once cells are in the S phase, cyclin E is degraded which may be required for terminating the S-phase initiation events, allowing the cells to progress from the S phase into the G2 phase (Clurman *et al.*, (1996) Genes Dev. 10, 1979-1990; Won *et al.*, (1996) EMBO J. 15, 4182-4193). Applicants have identified that SKP2 is also involved in the ubiquitin-dependent degradation of cyclin E and therefore the invention encompasses modulation of SKP2 activity and expression as a means of regulating cell cycle progression.

Applicants have determined that a number of phosphorylation dependent and ubiquitin-dependent degradation events occur during the G1/S transition, which are temporally regulated. The expression of SKP2 in the late G1 and S phases leads to assembly of the SCF<sup>SKP2</sup> complex. Previous reports suggest that the phosphorylation status of p27 and cyclin E could be temporally separated. p27 phosphorylation on the critical Thr187 has been shown to occur in the late G1 phase and p27 ubiquitination has been reported to require its binding to the cyclin E/CDK2 complex (Montagnoli *et al.*, (1999) Genes Dev. 13, 1181-1189). The phosphorylation of Thr187 in p27 triggers the binding of SKP2, leading to the subsequent ubiquitin-dependent degradation of p27.

It has been shown that binding of p27 to cyclin E/CDK complexes inhibits the activity of cyclin E/CDK2 and cyclin E degradation (Clurman *et al.*, (1996) Genes Dev. 10, 1979-1990). The binding of p27 therefore prevents phosphorylation on Thr380 in cyclin E or there is a competition between p27 and cyclin E for the binding of SKP2. Applicants have also demonstrated that p27 binding can also cause a conformational change in cyclin E so that Thr380 in cyclin E is not exposed for phosphorylation or SKP2 binding. Applicants have determined that SKP2 binds to the p27 phosphopeptide with higher affinity than that of cyclin E peptide. Thus the affinities between SKP2 and p27 or

cyclin E may also affect the ubiquitination rate of p27 and cyclin E by SKP2. Once p27 is degraded, the cyclin E/CDK2 kinase activity is activated, leading to the S-phase entry. Activation of cyclin E also leads to its autophosphorylation in Thr380 (Clurman *et al.*, (1996) *Genes Dev.* 10, 1979-1990; Won & Reed, (1996) *EMBO J.* 15, 4182-4193). The phosphorylation of Thr380 promotes the SKP2 binding which in turn results in the ubiquitin-dependent degradation of cyclin E. The invention therefore encompasses peptides capable of blocking the interaction of SKP2 and SKP2-like proteins with autologous and heterologous target proteins.

10 C. Alteration of Substrate-Specificity of Various F-box Proteins

F-box proteins are the substrate-targeting component of the SCF complex (SKP1, CUL-1, F-box proteins) (Zhang *et al.*, (1995) *Cell* 82, 915-925; Bai *et al.*, (1996) *Cell* 86, 263-274; Feldman *et al.*, (1997) *Cell* 91, 221-230; Skowyra *et al.*, (1997) *Cell* 91, 209-219). The F-box is a 40-50 amino-acid motif that is commonly present in the otherwise diverse proteins (Zhang *et al.*, (1995) *Cell* 82, 915-925; Bai *et al.*, (1996) *Cell* 86, 263-274). This motif mediates the interaction between an F-box protein and SKP1 (SEQ ID NO: 69). Applicants have identified about 30 F-box proteins which share no apparent homology except in the F-box motif (Figure 12). In mammals, two F-box proteins, SKP2 and  $\beta$ -TRCP, have been well characterized.

20 Applicants have also determined that SKP2 binds to p27, a CDK inhibitor, through the phosphorylated threonine187 and this interaction targets p27 for ubiquitin-dependent degradation. Applicants further determined that SKP2 interacts with and ubiquitinates cyclin E when the threonine380 of cyclin E is phosphorylated. Likewise it has been shown that  $\beta$ -TRCP (ZF11) binds to two critical serine residues in both  $\beta$ -catenin (serines 33 and 37) and I $\kappa$ B- $\alpha$  (serines 32 and 36) when they are phosphorylated (Maniatis, (1999) *Genes Dev.* 13, 505-510; Winston *et al.*, (1999) *Genes Dev.* 13, 270-283; Spencer *et al.*, (1999) *Genes Dev.* 13, 284-94; Yaron *et al.*, (1998) *Nature* 396, 590-594). This interaction leads to the ubiquitination and degradation of  $\beta$ -catenin or I $\kappa$ B- $\alpha$ . The difference in the substrate binding and thus the substrate specificity by these two F-box proteins is that SKP2 contains a substrate interaction domain of leucine-rich repeats (LRR) at its carboxy-terminal region (residues 220-400) (Zhang *et al.*, (1995) *Cell* 82, 915-25)



while  $\beta$ -TRCP (ZF11) instead has a completely different substrate-interaction domain consisting of WD40 repeats (WD) in the similar position (residues 212-569) (Winston *et al.*, (1999) *Genes Dev* 13, 270-283).

The substrate-specificity of these protein-protein interaction domains has been established through the analysis of yeast F-box proteins such as CDC4, a WD-repeat containing F-box protein, and GRR1, an F-box protein that has LRR at its carboxy terminus (Skowrya *et al.*, (1997) *Cell* 91, 209-219). Although the F-box proteins containing the LRR and WD repeats preferentially bind to substrates only when the substrates are phosphorylated, the existence of many F-box proteins that contain diverse protein-protein interaction domains indicates that many interact with target proteins directly without phosphorylation of the targets Winston *et al.*, (1999) *Curr. Biol.* 9, 1180-1182; Cenciarelli *et al.*, (1999) *Curr. Biol.* 9, 1177-1179).

The fact that various F-box proteins contain completely different substrate-interaction domains indicates that these domains are specifically used to contact substrates.

Once the substrates are in association with the F-box proteins, the presence of the F-box region in the F-box proteins promotes the binding of SKP1 and CUL-1 (SEQ ID NO: 71), as well as additional SCF components such as the recently identified Rbx1/Roc1&2 (Ohta *et al.*, (1999) *Mol. Cell.* 3, 535-541; Skowrya *et al.*, (1999) *Science* 284, 662-665; Kamura *et al.*, (1999) *Science* 284, 657-661), to form the SCF complexes. The assembly of the complete SCF ubiquitin E3 ligase complexes promotes the ubiquitin-transfer reaction to the SCF-interacting substrates by the ubiquitin conjugating E2 enzyme, CDC34, and the ubiquitin activating enzyme E1 (Koepp *et al.*, (1999) *Cell* 97, 431-434). The polyubiquitinated substrate proteins are subsequently degraded by the 26S proteasome.

In the case of SKP2 and  $\beta$ -TRCP, the effect of mutation in the F-box region has been examined. Expression of mutant forms of SKP2 or  $\beta$ -TRCP that contain a deletion in the F-box but retain the complete substrate-interaction domain of LRR or WD repeats causes the protection of their respective substrates, p27, cyclin E or  $\beta$ -catenin and I $\kappa$ B (Carrano *et al.*, (1999) *Nat. Cell. Biol.* 1, 193-199; Winston *et al.*, (1999) *Genes Dev.* 13, 270-283; Spencer *et al.*, (1999) *Genes Dev.* 13, 284-294) (Figure 9). This is because these SKP2 or  $\beta$ -TRCP mutants are fully capable of binding to the substrates while defective in

recruiting the SKP1/CUL-1 into the complex, producing a dominant negative effect for the stability of the target proteins *in vivo*.

The concept of altering the substrate specificity of the various F-box proteins can thus be extended to fuse a protein interaction domain or a ligand binding site, in the form of either a protein, a peptide, or a chemical, with the F-box motif of either SKP2,  $\beta$ -TRCP (ZF11) or other F-box proteins (ZF series). In this design, this hybrid protein or molecule can be used to bind its normal protein partner and targets the protein partner for ubiquitin-dependent degradation. For example, if the F-box protein is fused with Max or Mad, proteins that bind to Myc oncoprotein (Blackwood & Eisenman, (1991) Science 251, 1211-1217; Blackwood *et al.*, (1991) Cold Spring Harb. Symp. Quant. Biol. 56, 109-117), the F-box/Max or Mad fusion protein will bind to and target Myc for ubiquitination and degradation.

Thus in one aspect of the invention, the protein levels of Myc in a cell can be modulated by such an F-box/Max or Mad hybrid construct. Another example is fusion of the amino-terminus of MDM2 (residues 1-158), a region that is known to bind the tumor suppressor protein p53 (Chen *et al.*, (1993) Mol. Cell. Biol. 13, 4107-4114), with the F-box region derived from SKP2,  $\beta$ -TRCP and other F-box proteins. A hybrid F-box/MDM2 protein could be generated that would target p53 for ubiquitination. Such a pairwise selection can be extended to the cyclin-CDK (Hunter & Pines, (1994) Cell 79, 573-582), Bcl-2-Bax/Bad (Yang *et al.*, (1995) Cell 80, 285-291; Chao & Korsmeyer, (1998) Annu. Rev. Immunol. 16, 395-419), and many others for the selective degradation of the desired targets.

The concept of modulating protein levels by the alteration of SCF substrate-targeting specificity can be further extended to include fusing the protein-interaction domains with a peptide or a chemical that interact with SKP1 or CUL1 or the SCF complex. A fusion protein is an expression product resulting from the fusion of two genes. Such a protein may be produced, *e.g.*, in recombinant DNA expression studies or, naturally, in certain viral oncogenes in which the oncogene is fused to *gag*.

The production of a fusion protein sometimes results from the need to place a cloned eukaryotic gene under the control of a bacterial promoter for expression in a bacterial system. Sequences of the bacterial system are then frequently expressed linked

to the eukaryotic protein. Fusion proteins are used for the analysis of structure, purification, function, and expression of heterologous gene products.

A fused protein is a hybrid protein molecule which can be produced when a nucleic acid of interest is inserted by recombinant DNA techniques into a recipient plasmid and displaces the stop codon for a plasmid gene. The fused protein begins at the amino end with a portion of the plasmid protein sequence and ends with the protein of interest.

The production of fusion proteins is well known to one skilled in the art (see U.S. Patent Numbers 5,908,756; 5,907,085; 5,906,819; 5,905,146; 5,895,813; 5,891,643; 5,891,628; 5,891,432; 5,889,169; 5,889,150; 5,888,981; 5,888,773; 5,886,150; 5,886,149; 5,885,833; 5,885,803; 5,885,779; 5,885,580; 5,883,124; 5,882,941; 5,882,894; 5,882,864; 5,879,917; 5,879,893; 5,876,972; 5,874,304; and 5,874,290). For a general review of the construction, properties, applications and problems associated with specific types of fusion molecules used in clinical and research medicine, see Chamow *et al.*, (1999) Antibody Fusion Proteins, John Wiley.

#### D. Modulation of SKP2 Expression and Activity

The identification of SKP2 and SKP2-like proteins has led to the discovery of compounds that are capable of down-regulating expression of these proteins. Molecules that down-regulate SKP2 and SKP2-like proteins are therefore part of the invention.

Down-regulation is defined here as a decrease in activation, function or synthesis of SKP2 and SKP2-like proteins, its ligands or activators. It is further defined to include an increase in the degradation of the SKP2 gene, its protein product, ligands or activators.

Down-regulation is therefore achieved in a number of ways. For example, administration of molecules that can destabilize the binding of SKP2 and SKP2-like proteins with its

ligands. Such molecules encompass polypeptide products, including those encoded by the DNA sequences of the SKP2 gene or DNA sequences containing various mutations.

These mutations may be point mutations, insertions, deletions or spliced variants of the SKP2 gene. This invention also includes truncated polypeptides encoded by the DNA molecules described above. These polypeptides being capable of interfering with interaction of SKP2 and SKP2-like proteins with other proteins.

A further embodiment of this invention includes the down-regulation of SKP2 function by altering expression of the SKP2 gene, the use of antisense gene therapy being an example. Down-regulation of SKP2 or SKP2-like protein expression is accomplished by administering an effective amount of antisense oligonucleotides. These antisense  
5 molecules can be fashioned from the DNA sequence of the SKP2 gene or sequences containing various mutations, deletions, insertions or spliced variants. Another embodiment of this invention relates to the use of isolated RNA or DNA sequences derived from the SKP2 gene. These sequences containing various mutations such as point mutations, insertions, deletions or spliced variant mutations of SKP2 gene and can be  
10 useful in gene therapy.

Molecules that increase the degradation of the SKP2 or SKP2-like proteins may also be used to down-regulate its functions and are within the scope of the invention. Phosphorylation of SKP2 or SKP2-like proteins may alter protein stability, therefore kinase inhibitors may be used to down-regulate its function. Down-regulation of SKP2 or  
15 SKP2-like proteins may also be accomplished by the use of polyclonal or monoclonal antibodies or fragments thereof directed against the SKP2 or SKP2-like proteins. Such molecules are within the claimed invention. This invention further includes small molecules with the three-dimensional structure necessary to bind with sufficient affinity to block SKP2 or SKP2-like protein interactions with p27 or cyclin E. SKP2 or SKP2-like  
20 protein blockade resulting in decreased degradation of p27 or cyclin E and other processes of transformed cells where it is expressed make these small molecules useful as therapeutic agents in treating tumors.

The agents discussed above represent various effective therapeutic compounds in treating tumors. Applicants have thus provided antagonists and methods of identifying  
25 antagonists that are capable of down-regulating SKP2 or SKP2-like proteins.

A further embodiment of the invention relates to antisense or gene therapy. It is now known in the art that altered DNA molecules can be tailored to provide a specific selected effect, when provided as antisense or gene therapy. The native DNA segment coding for SKP2 has, as do all other mammalian DNA strands, two strands; a sense strand  
30 and an antisense strand held together by hydrogen bonds. The mRNA coding for SKP2 has a nucleotide sequence identical to the sense strand, with the expected substitution of

thymidine by uridine. Thus, based upon the knowledge of the SKP2 sequence, synthetic oligonucleotides can be synthesized. These oligonucleotides can bind to the DNA and RNA coding for SKP2. The active fragments of the invention, which are complementary to mRNA and the coding strand of DNA, are usually at least about 15 nucleotides, more  
5 usually at least 20 nucleotides, preferably 30 nucleotides and more preferably may be 50 nucleotides or more. There is no upper limit, other than a practical limit, on the maximal size of such a nucleic acid molecule in that the nucleic acid molecule can include a portion of a gene, an entire gene, or multiple genes, or portions thereof. The binding strength between the sense and antisense strands is dependent upon the total hydrogen bonds.  
10 Therefore, based upon the total number of bases in the mRNA, the optimal length of the oligonucleotide sequence may be easily calculated by the skilled artisan. The sequence may be complementary to any portion of the sequence of the mRNA. For example, it may be proximal to the 5'-terminus or capping site or downstream from the capping site, between the capping site and the initiation codon and may cover all or only a portion of  
15 the non-coding region or the coding region. The particular site(s) to which the antisense sequence binds will vary depending upon the degree of inhibition desired, the uniqueness of the sequence, the stability of the antisense sequence, etc.

In the practice of the invention, expression of SKP2 or SKP2-like proteins are down-regulated by administering an effective amount of synthetic antisense  
20 oligonucleotide sequences described above. The oligonucleotide compounds of the invention bind to the mRNA coding for human SKP2 thereby inhibiting expression (translation) of these proteins. The isolated DNA sequences containing various mutations such as point mutations, insertions, deletions or spliced mutations of SKP2 are useful in gene therapy as well.

25 Antisense oligonucleotides can also be used as tools *in vitro* to determine the biological function of genes and proteins. Oligonucleotide phosphorothioates (PS-oligos) have also shown great therapeutic potential as antisense-mediated inhibitors of gene expression. Various methods have been developed for the synthesis of antisense oligonucleotides. See Agrawal *et al.*, (1993) *Methods of Molecular Biology: Protocols for*  
30 *Oligonucleotides and Analogs*, Humana Press; Eckstein *et al.*, (1991) *Oligonucleotides and Analogues: A Practical Approach*, Oxford University Press).

### E. Diagnostic Assays

In another diagnostic embodiment, susceptibility to certain tumors associated with elevated levels of SKP2 or SKP2-like proteins in a human subject can be measured by the steps of: (a) measuring the level of SKP2 or SKP2-like proteins in a biological sample from said human subject; and (b) comparing the level of SKP2 or SKP2-like proteins present in normal subjects, wherein an increase in the level of SKP2 or SKP2-like proteins as compared to normal levels indicates a predisposition to certain tumors.

In another diagnostic embodiment, a therapeutic treatment of certain tumors associated with elevated levels of SKP2 or SKP2-like proteins in a human subject may be monitored by measuring the levels of SKP2 or SKP2-like proteins in a series of biologic samples obtained at different time points from said subject undergoing therapeutic treatment wherein a significant decrease in said levels of SKP2 or SKP2-like proteins indicates a successful therapeutic treatment.

Diagnostic probes useful in such assays of the invention include antibodies to SKP2 or SKP2-like proteins. The antibodies to SKP2 or SKP2-like proteins may be either monoclonal or polyclonal, produced using standard techniques well known in the art (See Harlow & Lane, (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press). They can be used to detect SKP2 or SKP2-like proteins by binding to the protein and subsequent detection of the antibody-protein complex by ELISA, Western blot or the like. The SKP2 or SKP2-like proteins used to elicit these antibodies can be any of the SKP2 or SKP2-like proteins variants discussed above. Antibodies are also produced from peptide sequences of SKP2 or SKP2-like proteins using standard techniques in the art (See Protocols in Immunology, John Wiley & Sons, 1994). Fragments of the monoclonals or the polyclonal antisera which contain the immunologically significant portion can also be prepared. Use of immunologically reactive fragments, such as the Fab, Fab', of F(ab')<sub>2</sub> fragments is often preferable, especially in a therapeutic context, as these fragments are generally less immunogenic than the whole immunoglobulin.

Assays to detect or measure SKP2 or SKP2-like proteins polypeptide in a biological sample with an antibody probe may be based on any available format. For instance, in immunoassays where SKP2 or SKP2-like proteins are the analyte, the test

sample, typically a biological sample, is incubated with anti-SKP2 antibodies under conditions that allow the formation of antigen-antibody complexes. Various formats can be employed, such as "sandwich" assay where antibody bound to a solid support is incubated with the test sample; washed, incubated with a second, labeled antibody to the  
5 analyte; and the support is washed again. Analyte is detected by determining if the second antibody is bound to the support. In a competitive format, which can be either heterogeneous or homogeneous, a test sample is usually incubated with an antibody and a labeled competing antigen, either sequentially or simultaneously. These and other formats are well known in the art.

10

#### F. Methods to Identify Binding Partners

Another embodiment of the present invention provides methods for use in isolating and identifying binding partners of proteins of the invention. In detail, a protein of the invention is mixed with a potential binding partner or an extract or fraction of a cell under  
15 conditions that allow the association of potential binding partners with the protein of the invention. After mixing, peptides, polypeptides, proteins or other molecules that have become associated with a protein of the invention are separated from the mixture. The binding partner bound to the protein of the invention can then be removed and further analyzed. To identify and isolate a binding partner, the entire protein, for instance the  
20 entire SKP2 or SKP2-like protein can be used. Alternatively, a fragment of the protein can be used, such as the SKP-1 interacting domain.

As used herein, a cellular extract refers to a preparation or fraction which is made from a lysed or disrupted cell. The preferred source of cellular extracts will be cells derived from human tissue, for instance, malignant tissue. Alternatively, cellular extracts  
25 may be prepared from any source of malignant tissue or available cell lines.

A variety of methods can be used to obtain an extract of a cell. Cells can be disrupted using either physical or chemical disruption methods. Examples of physical disruption methods include, but are not limited to, sonication and mechanical shearing. Examples of chemical lysis methods include, but are not limited to, detergent lysis and  
30 enzyme lysis. A skilled artisan can readily adapt methods for preparing cellular extracts in order to obtain extracts for use in the present methods.

Once an extract of a cell is prepared, the extract is mixed with the protein of the invention under conditions in which association of the protein with the binding partner can occur. A variety of conditions can be used, the most preferred being conditions that closely resemble conditions found in the cytoplasm of a human cell. Features such as osmolarity, pH, temperature, and the concentration of cellular extract used, can be varied to optimize the association of the protein with the binding partner.

After mixing under appropriate conditions, the bound complex is separated from the mixture. A variety of techniques can be utilized to separate the mixture. For example, antibodies specific to a protein of the invention can be used to immunoprecipitate the binding partner complex. Alternatively, standard chemical separation techniques such as chromatography and density-sediment centrifugation can be used.

After removal of non-associated cellular constituents found in the extract, the binding partner can be dissociated from the complex using conventional methods. For example, dissociation can be accomplished by altering the salt concentration or pH of the mixture.

To aid in separating associated binding partner pairs from the mixed extract, the protein of the invention can be immobilized on a solid support. For example, the protein can be attached to a nitrocellulose matrix or acrylic beads. Attachment of the protein to a solid support aids in separating peptide-binding partner pairs from other constituents found in the extract. The identified binding partners can be either a single protein or a complex made up of two or more proteins. Alternatively, binding partners may be identified using the Alkaline Phosphatase fusion assay according to the procedures of Flanagan & Vanderhaeghen, (1998) *Annu. Rev. Neurosci.* 21, 309-345 or Takahashi *et al.*, (1999) *Cell* 99, 59-69; the Far-Western assay according to the procedures of Takayama *et al.*, (1997) *Methods Mol. Biol.* 69, 171-184 or Sauder *et al.*, *J. Gen. Virol.* (1996) 77, 991-996 or identified through the use of epitope tagged proteins or GST fusion proteins.

Alternatively, the nucleic acid molecules of the invention can be used in a yeast two-hybrid system. The yeast two-hybrid system has been used to identify other protein partner pairs and can readily be adapted to employ the nucleic acid molecules herein described (see Stratagene Hybrizap® two-hybrid system).



### G. Methods to Identify Agents that Modulate Expression

Another embodiment of the present invention provides methods for identifying agents that modulate the expression of a nucleic acid encoding the SKP2 protein, or of a nucleic acid encoding the SKP2 or SKP2-like protein such as a protein. Such assays may  
5 utilize any available means of monitoring for changes in the expression level of the nucleic acids of the invention. As used herein, an agent is said to modulate the expression of a nucleic acid, for instance a nucleic acid encoding the protein having the sequence of SKP2, SKP2-like proteins, SKP1, CUL-1, or any F-box containing protein such as a ZF protein, if it is capable of up- or down-regulating expression of the nucleic acid in a cell.

10 In one assay format, cell lines that contain reporter gene fusions between the open reading frame of SKP2 or a SKP2-like protein and any assayable fusion partner may be prepared. Numerous assayable fusion partners are known and readily available including the firefly luciferase gene and the gene encoding chloramphenicol acetyltransferase (Alam *et al.*, (1990) Anal. Biochem. 188, 245-254). Cell lines containing the reporter gene  
15 fusions are then exposed to the agent to be tested under appropriate conditions and time. Differential expression of the reporter gene between samples exposed to the agent and control samples identifies agents which modulate the expression of a nucleic acid encoding an SKP2, SKP2-like or ZF protein.

Additional assay formats may be used to monitor the ability of the agent to  
20 modulate the expression of a nucleic acid encoding a SKP-2 or SKP2-like protein. For instance, mRNA expression may be monitored directly by hybridization to the nucleic acids of the invention. Cell lines are exposed to the agent to be tested under appropriate conditions and time and total RNA or mRNA is isolated by standard procedures such those disclosed in Sambrook *et al.*, (1989) Molecular Cloning - A Laboratory Manual, Cold  
25 Spring Harbor Laboratory Press.

Probes to detect differences in RNA expression levels between cells exposed to the agent and control cells may be prepared from the nucleic acids of the invention. It is preferable, but not necessary, to design probes which hybridize only with target nucleic acids under conditions of high stringency. Only highly complementary nucleic acid  
30 hybrids form under conditions of high stringency. Accordingly, the stringency of the assay conditions determines the amount of complementarity which should exist between

two nucleic acid strands in order to form a hybrid. Stringency should be chosen to maximize the difference in stability between the probe:target hybrid and potential probe:non-target hybrids.

5 Probes may be designed from the nucleic acids of the invention through methods known in the art. For instance, the G+C content of the probe and the probe length can affect probe binding to its target sequence. Methods to optimize probe specificity are commonly available in Sambrook *et al.*, (1989) Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratory Press or Ausubel *et al.*, (1995) Current Protocols in Molecular Biology, Greene Publishing.

10 Hybridization conditions are modified using known methods, such as those described by Sambrook *et al.*, (1989) and Ausubel *et al.*, (1995) as required for each probe. Hybridization of total cellular RNA or RNA enriched for polyA+ RNA can be accomplished in any available format. For instance, total cellular RNA or RNA enriched for polyA+ RNA can be affixed to a solid support and the solid support exposed to at least  
15 one probe comprising at least one, or part of one of the sequences of the invention under conditions in which the probe will specifically hybridize. Alternatively, nucleic acid fragments comprising at least one, or part of one of the sequences of the invention can be affixed to a solid support, such as a silicon based wafer or a porous glass wafer. The wafer can then be exposed to total cellular RNA or polyA+ RNA from a sample under  
20 conditions in which the affixed sequences will specifically hybridize. Such wafers and hybridization methods are widely available, for example, those disclosed by Beattie, (WO9511755). By examining for the ability of a given probe to specifically hybridize to a RNA sample from an untreated cell population and from a cell population exposed to the agent, agents which up or down regulate the expression of a nucleic acid encoding the  
25 SKP2 protein are identified.

Hybridization for qualitative and quantitative analysis of mRNA may also be carried out by using a RNase Protection Assay (*i.e.*, RPA, see Ma *et al.*, Methods (1996) 10, 273-238). Briefly, an expression vehicle comprising cDNA encoding the gene product and a phage specific DNA dependent RNA polymerase promoter (*e.g.*, T7, T3 or SP6  
30 RNA polymerase) is linearized at the 3' end of the cDNA molecule, downstream from the phage promoter, wherein such a linearized molecule is subsequently used as a template for

synthesis of a labeled antisense transcript of the cDNA by *in vitro* transcription. The labeled transcript is then hybridized to a mixture of isolated RNA (*i.e.*, total or fractionated mRNA) by incubation at 45°C overnight in a buffer comprising 80% formamide, 40 mM Pipes, pH 6.4, 0.4 M NaCl and 1 mM EDTA. The resulting hybrids are then digested in a buffer comprising 40 µg/ml ribonuclease A and 2 µg/ml ribonuclease. After deactivation and extraction of extraneous proteins, the samples are loaded onto urea-polyacrylamide gels for analysis.

In another assay format, agents which effect the expression of the instant gene products, cells or cell lines would first be identified which express said gene products physiologically. Cells and cell lines so identified would be expected to comprise the necessary cellular machinery such that the fidelity of modulation of the transcriptional apparatus is maintained with regard to exogenous contact of agent with appropriate surface transduction mechanisms and the cytosolic cascades. Further, such cells or cell lines would be transduced or transfected with an expression vehicle (*e.g.*, a plasmid or viral vector) construct comprising an operable non-translated 5'-promoter containing end of the structural gene encoding the instant gene products fused to one or more antigenic fragments, which are peculiar to the instant gene products, wherein said fragments are under the transcriptional control of said promoter and are expressed as polypeptides whose molecular weight can be distinguished from the naturally occurring polypeptides or may further comprise an immunologically distinct tag. Such a process is well known in the art (see, Sambrook *et al.*, (1989) Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratory Press).

Cells or cell lines transduced or transfected as outlined above would then be contacted with agents under appropriate conditions; for example, the agent comprises a pharmaceutically acceptable excipient and is contacted with cells in an aqueous physiological buffer such as phosphate buffered saline (PBS) at physiological pH, Eagles balanced salt solution (BSS) at physiological pH, PBS or BSS comprising serum or conditioned media comprising PBS or BSS and serum incubated at 37°C. Said conditions may be modulated as deemed necessary by one of skill in the art. Subsequent to contacting the cells with the agent, said cells will be disrupted and the polypeptides of the disruptate are fractionated such that a polypeptide fraction is pooled and contacted with an antibody

to be further processed by immunological assay (e.g., ELISA, immunoprecipitation or Western blot). The pool of proteins isolated from the "agent contacted" sample will be compared with a control sample where only the excipient is contacted with the cells and an increase or decrease in the immunologically generated signal from the "agent contacted" sample compared to the control will be used to distinguish the effectiveness of the agent.

#### H. Methods to Identify Agents that Modulate Activity

Another embodiment of the present invention provides methods for identifying agents that modulate at least one activity of a protein of the invention such as SKP2 or SKP2-like proteins. Such methods or assays may utilize any means of monitoring or detecting the desired activity.

The present invention includes methods of screening for compounds which deactivate, or act as antagonists of SKP2 or SKP2-like protein expression. Such compounds may be useful in the modulation of pathological conditions associated with alterations in SKP2, SKP2-like or p27 protein levels.

In one format, the relative amounts of a SKP2 protein between a cell population that has been exposed to the agent to be tested compared to an un-exposed control cell population may be assayed. In this format, probes such as specific antibodies are used to monitor the differential expression of the protein in the different cell populations. Cell lines or populations are exposed to the agent to be tested under appropriate conditions and time. Cellular lysates may be prepared from the exposed cell line or population and a control, unexposed cell line or population. The cellular lysates are then analyzed with the probe.

Antibody probes are prepared by immunizing suitable mammalian hosts in appropriate immunization protocols using the SKP2 or SKP2-like proteins if they are of sufficient length, or if desired, or if required to enhance immunogenicity, conjugated to suitable carriers. Methods for preparing immunogenic conjugates with carriers such as BSA, KLH, or other carrier proteins are well known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents may be effective; in other instances linking reagents such as those supplied by Pierce Chemical Co. may be desirable to provide accessibility to the hapten. The hapten peptides can be extended at either the

amino or carboxy terminus with a cysteine residue or interspersed with cysteine residues, for example, to facilitate linking to a carrier. Administration of the immunogens is conducted generally by injection over a suitable time period and with use of suitable adjuvants, as is generally understood in the art. During the immunization schedule, titers  
5 of antibodies are taken to determine adequacy of antibody formation.

While the polyclonal antisera produced in this way may be satisfactory for some applications, for pharmaceutical compositions, use of monoclonal preparations is preferred. Immortalized cell lines which secrete the desired monoclonal antibodies may be prepared using the standard method of Kohler & Milstein, (1992) *Biotechnology* 24, 524-  
10 526 or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known. The immortalized cell lines secreting the desired antibodies are screened by immunoassay in which the antigen is the peptide hapten, polypeptide or protein. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells can be cultured either *in vitro* or by production in ascites fluid.

15 The desired monoclonal antibodies may be recovered from the culture supernatant or from the ascites supernatant. Fragments of the monoclonals or the polyclonal antisera which contain the immunologically significant portion can be used as antagonists, as well as the intact antibodies. Use of immunologically reactive fragments, such as the Fab, Fab' of F(ab')<sub>2</sub> fragments is often preferable, especially in a therapeutic context, as these  
20 fragments are generally less immunogenic than the whole immunoglobulin.

The antibodies or fragments may also be produced, using current technology, by recombinant means. Antibody regions that bind specifically to the desired regions of the protein can also be produced in the context of chimeras with multiple species origin.

25 Antibody regions that bind specifically to the desired regions of the protein can also be produced in the context of chimeras with multiple species origin, for instance, humanized antibodies. The antibody can therefore be a humanized antibody or human a antibody, as described in U. S. Patent No. 5,585,089 or Riechmann *et al.*, (1988) *Nature* 332, 323-327.

30 Agents that are assayed in the above method can be randomly selected or rationally selected or designed. As used herein, an agent is said to be randomly selected when the agent is chosen randomly without considering the specific sequences involved in the

association of the a protein of the invention alone or with its associated substrates, binding partners, etc. An example of randomly selected agents is the use a chemical library or a peptide combinatorial library, or a growth broth of an organism.

As used herein, an agent is said to be rationally selected or designed when the  
5 agent is chosen on a non-random basis which takes into account the sequence of the target site or its conformation in connection with the agent's action. Agents can be rationally selected or rationally designed by utilizing the peptide sequences that make up these sites. For example, a rationally selected peptide agent can be a peptide whose amino acid  
10 sequence is identical to the SKP1 or SKP2 interaction domain on a autologous or heterologous target protein which interacts with the SKP2 protein or its targets.

The agents of the present invention can be, as examples, peptides, small molecules, vitamin derivatives, as well as carbohydrates. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents of the present invention.

The peptide agents of the invention can be prepared using standard solid phase (or  
15 solution phase) peptide synthesis methods, as is known in the art. In addition, the DNA encoding these peptides may be synthesized using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production using solid phase peptide synthesis is necessitated if non-gene-encoded amino acids are to be included.

20 Another class of agents of the present invention are antibodies immunoreactive with critical positions of proteins of the invention. For example, antibodies which specifically interact with the SKP1 interacting domain, SKP2 interacting domain or the SKP2 C-terminal motif. Antibody agents are obtained by immunization of suitable mammalian subjects with peptides, containing as antigenic regions, those portions of the  
25 protein intended to be targeted by the antibodies.

### I. High Throughput Assays

The power of high throughput screening is utilized to the search for new  
compounds which are capable of interacting with the SKP2 or SKP-2 like proteins. For  
30 general information on high-throughput screening (see Devlin, (1998) High Throughput

Screening, Marcel Dekker; U.S. Patent No. 5,763,263). High throughput assays utilize one or more different assay techniques.

Immunodiagnosics and Immunoassays. These are a group of techniques used for the measurement of specific biochemical substances, commonly at low concentrations in complex mixtures such as biological fluids, that depend upon the specificity and high affinity shown by suitably prepared and selected antibodies for their complementary antigens. A substance to be measured must, of necessity, be antigenic - either an immunogenic macromolecule or a haptenic small molecule. To each sample a known, limited amount of specific antibody is added and the fraction of the antigen combining with it, often expressed as the bound:free ratio, is estimated, using as indicator a form of the antigen labeled with radioisotope (radioimmunoassay), fluorescent molecule (fluoroimmunoassay), stable free radical (spin immunoassay), enzyme (enzyme immunoassay), or other readily distinguishable label.

Antibodies can be labeled in various ways, including: enzyme-linked immunosorbent assay (ELISA); radioimmuno assay (RIA); fluorescent immunoassay (FIA); chemiluminescent immunoassay (CLIA); and labeling the antibody with colloidal gold particles (immunogold).

Common assay formats include the sandwich assay, competitive or competition assay, latex agglutination assay, homogeneous assay, microtitre plate format and the microparticle-based assay.

Enzyme-linked immunosorbent assay (ELISA). ELISA is an immunochemical technique that avoids the hazards of radiochemicals and the expense of fluorescence detection systems. Instead, the assay uses enzymes as indicators. ELISA is a form of quantitative immunoassay based on the use of antibodies (or antigens) that are linked to an insoluble carrier surface, which is then used to "capture" the relevant antigen (or antibody) in the test solution. The antigen-antibody complex is then detected by measuring the activity of an appropriate enzyme that had previously been covalently attached to the antigen (or antibody).

For information on ELISA techniques, see, for example, Crowther, (1995) ELISA - Theory and Practice (Methods in Molecular Biology), Humana Press; Challacombe & Kemeny, (1998) ELISA and Other Solid Phase Immunoassays - Theoretical and Practical

Aspects, John Wiley; Kemeny, (1991) A Practical Guide to ELISA, Pergamon Press; Ishikawa, (1991) Ultrasensitive and Rapid Enzyme Immunoassay (Laboratory Techniques in Biochemistry and Molecular Biology) Elsevier.

Colorimetric Assays for Enzymes. Colorimetry is any method of quantitative chemical analysis in which the concentration or amount of a compound is determined by comparing the color produced by the reaction of a reagent with both standard and test amounts of the compound, often using a colorimeter. A colorimeter is a device for measuring color intensity or differences in color intensity, either visually or photoelectrically.

Standard colorimetric assays of beta-galactosidase enzymatic activity are well known to those skilled in the art (see, for example, Norton *et al.*, (1985) Mol. Cell. Biol. 5, 281-290). A colorimetric assay can be performed on whole cell lysates using O-nitrophenyl-beta-D-galactopyranoside (ONPG, Sigma) as the substrate in a standard colorimetric beta-galactosidase assay (Sambrook *et al.*, (1989) Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratory Press. Automated colorimetric assays are also available for the detection of beta-galactosidase activity, as described in U.S. Patent No. 5,733,720.

Immunofluorescence Assays. Immunofluorescence or immunofluorescence microscopy is a technique in which an antigen or antibody is made fluorescent by conjugation to a fluorescent dye and then allowed to react with the complementary antibody or antigen in a tissue section or smear. The location of the antigen or antibody can then be determined by observing the fluorescence by microscopy under ultraviolet light.

For general information on immunofluorescent techniques, see, for example, Knapp *et al.*, (1978) Immunofluorescence and Related Staining Techniques, Elsevier; Allan, (1999) Protein Localization by Fluorescent Microscopy - A Practical Approach (The Practical Approach Series) Oxford University Press; Caul, (1993) Immunofluorescence Antigen Detection Techniques in Diagnostic Microbiology, Cambridge University Press. For detailed explanations of immunofluorescent techniques applicable to the present invention, see U.S. Patent Nos. 5,912,176; 5,869,264; 5,866,319; 5,861,259.



#### J. Pharmaceutical preparations

The invention also includes pharmaceutical compositions comprising the compounds of the invention together with a pharmaceutically acceptable carrier.

Pharmaceutically acceptable carriers can be sterile liquids, such as water and oils,

5 including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in Remington's  
10 Pharmaceutical Sciences, Mack Publishing Company, 1995. In addition to the pharmacologically active agent, the compositions of the present invention may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically for delivery to the site of action. Suitable formulations for parenteral  
15 administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain  
20 substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers. Liposomes can also be used to encapsulate the agent for delivery into the cell.

The pharmaceutical formulation for systemic administration according to the  
25 invention may be formulated for enteral, parenteral or topical administration. Indeed, all three types of formulations may be used simultaneously to achieve systemic administration of the active ingredient.

Suitable formulations for oral administration include hard or soft gelatin capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and  
30 controlled release forms thereof.

The agents of the present invention can be administered via parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, or buccal routes. Alternatively, or concurrently, administration may be by the oral route or directly to the lungs. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

The compounds used in the method of treatment of this invention may be administered systemically or topically, depending on such considerations as the condition to be treated, need for site-specific treatment, quantity of drug to be administered and similar considerations.

Topical administration may be used. Any common topical formation such as a solution, suspension, gel, ointment or salve and the like may be employed. Preparation of such topical formulations are well described in the art of pharmaceutical formulations as exemplified, for example, by Remington's Pharmaceutical Sciences. For topical application, these compounds could also be administered as a powder or spray, particularly in aerosol form. The active ingredient may be administered in pharmaceutical compositions adapted for systemic administration. As is known, if a drug is to be administered systemically, it may be confected as a powder, pill, tablet or the like or as a syrup or elixir for oral administration. For intravenous, intraperitoneal or intra-lesional administration, the compound will be prepared as a solution or suspension capable of being administered by injection. In certain cases, it may be useful to formulate these compounds in suppository form or as an extended release formulation for deposit under the skin or intramuscular injection. In a preferred embodiment, the compounds of this invention may be administered by inhalation. For inhalation therapy the compound may be in a solution useful for administration by metered dose inhalers or in a form suitable for a dry powder inhaler.

An effective amount is that amount which will modulate the activity or alter the level of a target protein. A given effective amount will vary from condition to condition and in certain instances may vary with the severity of the condition being treated and the patient's susceptibility to treatment. Accordingly, a given effective amount will be best determined at the time and place through routine experimentation. However, it is

anticipated that in the treatment of a tumor in accordance with the present invention, a formulation containing between 0.001 and 5 percent by weight, preferably about 0.01 to 1 percent, will usually constitute a therapeutically effective amount. When administered systemically, an amount between 0.01 and 100 mg per kg body weight per day, but preferably about 0.1 to 10 mg/kg, will effect a therapeutic result in most instances.

In practicing the methods of this invention, the compounds of this invention may be used alone or in combination, or in combination with other therapeutic or diagnostic agents. In certain preferred embodiments, the compounds of this invention may be coadministered along with other compounds typically prescribed for these conditions according to generally accepted medical practice. The compounds of this invention can be utilized *in vivo*, ordinarily in mammals, preferably in humans.

In still another embodiment, the compounds of the invention may be coupled to chemical moieties, including proteins that alter the functions or regulation of target proteins for therapeutic benefit. These proteins may include in combination other inhibitors of cytokines and growth factors that may offer additional therapeutic benefit in the treatment of tumors. In addition, the molecules of the invention may also be conjugated through phosphorylation to biotinylate, thioate, acetylate, iodinate using any of the cross-linking reagents well known in the art.

#### K. Transgenic Animals

The term "animal" as used herein includes all vertebrate animals, except humans. It also includes an individual animal in all stages of development, including embryonic and fetal stages. A "transgenic animal" is an animal containing one or more cells bearing genetic information received, directly or indirectly, by deliberate genetic manipulation at a subcellular level, such as by microinjection or infection with recombinant virus. This introduced DNA molecule may be integrated within a chromosome, or it may be extra-chromosomally replicating DNA. The term "germ cell-line transgenic animal" refers to a transgenic animal in which the genetic information was introduced into a germ line cell, thereby conferring the ability to transfer the information to offspring. If such offspring in fact possess some or all of that information, then they, too, are transgenic animals. Transgenic animals containing mutant, knock-out, modified genes or gene

constructs to over-express or conditionally express a gene corresponding to the cDNA sequence of SEQ ID NO: 66 or related sequences are encompassed in the invention.

The information may be foreign to the species of animal to which the recipient belongs, foreign only to the particular individual recipient, or genetic information already  
5 possessed by the recipient. In the last case, the introduced gene may be differently expressed compared to the native endogenous gene. The genes may be obtained by isolating them from genomic sources, by preparation of cDNA from isolated RNA templates, by directed synthesis, or by some combination thereof.

To be expressed, a gene should be operably linked to a regulatory region.  
10 Regulatory regions, such as promoters, may be used to increase, decrease, regulate or designate to certain tissues or to certain stages of development the expression of a gene. The promoter need not be a naturally occurring promoter. The "transgenic non-human animals" of the invention are produced by introducing "transgenes" into the germline of the non-human animal. The methods enabling the introduction of DNA into cells are  
15 generally available and well-known in the art. Different methods of introducing transgenes could be used. Generally, the zygote is the best target for microinjection. In the mouse, the male pronucleus reaches the size of approximately twenty microns in diameter, which allows reproducible injection of one to two picoliters of DNA solution. The use of zygotes as a target for gene transfer has a major advantage. In most cases, the  
20 injected DNA will be incorporated into the host gene before the first cleavage (Brinster *et al.*, (1985) Proc. Natl. Acad. Sci. USA 82, 4438-4442.). Consequently, nearly all cells of the transgenic non-human animal will carry the incorporated transgene. Generally, this will also result in the efficient transmission of the transgene to offspring of the founder since 50% of the germ cells will harbor the transgene. Microinjection of zygotes is a  
25 preferred method for incorporating transgenes in practicing the invention.

Retroviral infection can also be used to introduce a transgene into a non-human animal. The developing non-human embryo can be cultured *in vitro* to the blastocyst stage. During this time, blastomeres may be targets for retroviral infection. Efficient infection of the blastomeres is obtained by enzymatic treatment to remove the zona  
30 pellucida. The viral vector system used to introduce the transgene is typically a replication-defective retrovirus carrying the transgene (Jahner *et al.*, (1985) Proc. Natl.

Acad. Sci. USA 82, 6927-6931; Van der Putten *et al.*, (1985) Proc. Natl. Acad. Sci. USA 82, 6148-6152). Transfection is easily and efficiently obtained by culturing the blastomeres on a monolayer of virus-producing cells (Van der Putten *et al.*, (1985) Proc. Natl. Acad. Sci. USA 82, 6148-6152; Stewart *et al.*, (1987) EMBO J. 6, 383-388).

5 Alternatively, infection can be performed at a later stage. Virus or virus-producing cells can be injected into the blastocoele (Jahner *et al.*, (1982) Nature 298, 623-628). Most of the founder animals will be mosaic for the transgene since incorporation occurs only in a subset of the cells which formed the transgenic non-human animal. Furthermore, the founder animal may contain retroviral insertions of the transgene at a variety of positions  
10 in the genome; these generally segregate in the offspring. In addition, it is also possible to introduce transgenes into the germ line, albeit with low efficiency, by intrauterine retroviral infection of the midgestation embryo (Jahner *et al.*, (1982) Nature 298, 623-628).

A third type of target cell for transgene introduction is the embryonal stem cell  
15 (ES). ES cells are obtained from pre-implantation embryos cultured *in vitro* (Evans *et al.*, (1981) Nature 292, 154-156; Bradley *et al.*, (1984) Nature 309, 255-256; Gossler *et al.*, (1986) Proc. Natl. Acad. Sci. USA 83, 9065-9069). Transgenes can be efficiently introduced into ES cells by DNA transfection or by retrovirus-mediated transduction. The resulting transformed ES cells can thereafter be combined with blastocysts from a  
20 non-human animal. The ES cells colonize the embryo and contribute to the germ line of the resulting chimeric animal.

The methods for evaluating the presence of the introduced DNA as well as its expression are readily available and well-known in the art. Such methods include, but are not limited to DNA (Southern) hybridization to detect the exogenous DNA, polymerase  
25 chain reaction (PCR), polyacrylamide gel electrophoresis (PAGE) and Western blots to detect DNA, RNA and protein. The methods include immunological and histochemical techniques to detect expression of a gene.

As used herein, a "transgene" is a DNA sequence introduced into the germline of a non-human animal by way of human intervention such as by way of the Examples  
30 described below. The nucleic acid sequence of the transgene, in this case a form of SEQ ID NO: 66, may be integrated either at a locus of a genome where that particular nucleic

acid sequence is not otherwise normally found or at the normal locus for the transgene. The transgene may consist of nucleic acid sequences derived from the genome of the same species or of a different species than the species of the target animal.

As discussed above, a "vector" is any means for the transfer of a nucleic acid into a host cell. Preferred vectors are plasmids and viral vectors, such as retroviruses. Viral vectors may be used to produce a transgenic animal according to the invention. Preferably, the viral vectors are replication defective, that is, they are unable to replicate autonomously in the target cell. In general, the genome of the replication defective viral vectors which are used within the scope of the present invention lack at least one region which is necessary for the replication of the virus in the infected cell. These regions can either be eliminated (in whole or in part), or be rendered non-functional by any technique known to a person skilled in the art. These techniques include the total removal, substitution (by other sequences, in particular by the inserted nucleic acid), partial deletion or addition of one or more bases to an essential (for replication) region. Such techniques may be performed *in vitro* (on the isolated DNA) or *in situ*, using the techniques of genetic manipulation or by treatment with mutagenic agents.

Preferably, the replication defective virus retains the sequences of its genome which are necessary for encapsidating the viral particles. The retroviruses are integrating viruses which infect dividing cells. The retrovirus genome includes two LTRs, an encapsidation sequence and three coding regions (*gag*, *pol* and *env*). The construction of recombinant retroviral vectors has been described (see, for example, Bernstein *et al.*, (1985) Genet. Eng. 7, 235; McCormick, (1985) Biotechnol. 3, 689-691). In recombinant retroviral vectors, the *gag*, *pol* and *env* genes are generally deleted, in whole or in part, and replaced with a heterologous nucleic acid sequence of interest. These vectors can be constructed from different types of retrovirus, such as, HIV, MoMuLV (murine Moloney leukemia virus), MSV (murine Moloney sarcoma virus), HaSV (Harvey sarcoma virus); SNV (spleen necrosis virus); RSV (Rous sarcoma virus) and Friend virus.

In general, in order to construct recombinant retroviruses containing a nucleic acid sequence, a plasmid is constructed which contains the LTRs, the encapsidation sequence and the coding sequence. This construct is used to transfect a packaging cell line, which cell line is able to supply in trans the retroviral functions which are deficient in the

plasmid. In general, the packaging cell lines are thus able to express the *gag*, *pol* and *env* genes. Such packaging cell lines have been described in the prior art, in particular the cell line PA317 (U.S. Patent No. 4,861,719); the PsiCRIP cell line (WO9002806) and the GP+envAm-12 cell line (WO8907150). In addition, the recombinant retroviral vectors can  
5 contain modifications within the LTRs for suppressing transcriptional activity as well as extensive encapsidation sequences which may include a part of the *gag* gene (Bender *et al.*, (1987) J. Virol. 61, 1639-1646). Recombinant retroviral vectors are purified by standard techniques known to those having ordinary skill in the art.

In one aspect the nucleic acid encodes antisense RNA molecules. In this  
10 embodiment, the nucleic acid is operably linked to suitable regulatory regions (discussed above) enabling expression of the nucleic acid sequence, and is introduced into a cell utilizing, preferably, recombinant vector constructs, which will express the antisense nucleic acid once the vector is introduced into the cell. Examples of suitable vectors includes plasmids, adenoviruses, adeno-associated viruses (see, for example, U.S. Patent  
15 No. 4,797,368, U.S. Patent No. 5,139,941), retroviruses (see above), and herpes viruses. For delivery of a therapeutic gene the vector is preferably an adeno-associated virus.

Adenoviruses are eukaryotic DNA viruses that can be modified to efficiently deliver a nucleic acid of the invention to a variety of cell types. Various serotypes of adenovirus exist. Of these serotypes, preference is given, within the scope of the present  
20 invention, to using type two or type five human adenoviruses (Ad 2 or Ad 5) or adenoviruses of animal origin (see WO9426914). Those adenoviruses of animal origin which can be used within the scope of the present invention include adenoviruses of canine, bovine, murine, ovine, porcine, avian, and simian origin.

The replication defective recombinant adenoviruses according to the invention can  
25 be prepared by any technique known to the person skilled in the art. In particular, they can be prepared by homologous recombination between an adenovirus and a plasmid which carries, inter alia, the DNA sequence of interest. The homologous recombination is effected following cotransfection of the said adenovirus and plasmid into an appropriate cell line. The cell line which is employed should preferably (i) be transformable by the  
30 said elements, and (ii) contain the sequences which are able to complement the part of the genome of the replication defective adenovirus, preferably in integrated form in order to

avoid the risks of recombination. Recombinant adenoviruses are recovered and purified using standard molecular biological techniques, which are well known to one of ordinary skill in the art.

5 A number of recombinant or transgenic mice have been produced, including those which express an activated oncogene sequence (U.S. Patent No. 4,736,866); express Simian SV 40 T-antigen (U.S. Patent No. 5,728,915); lack the expression of interferon regulatory factor 1 (IRF-1) (U.S. Patent No. 5,731,490); exhibit dopaminergic dysfunction (U.S. Patent No. 5,723,719); express at least one human gene which participates in blood pressure control (U.S. Patent No. 5,731,489); display greater similarity to the conditions  
10 existing in naturally occurring Alzheimer's disease (U.S. Patent No. 5,720,936); have a reduced capacity to mediate cellular adhesion (U.S. Patent No. 5,602,307); possess a bovine growth hormone gene (Clutter *et al.*, (1996) Genetics 143, 1753-1760) or are capable of generating a fully human antibody response (Zou *et al.*, (1993) Science 262, 1271-1274).

15 While mice and rats remain the animals of choice for most transgenic experimentation, in some instances it is preferable or even necessary to use alternative animal species. Transgenic procedures have been successfully utilized in a variety of non-murine animals, including sheep, goats, chickens, hamsters, rabbits, cows and guinea pigs (see Aigner *et al.*, (1999) Biochem. Biophys. Res. Commun. 257, 843-850; Castro *et al.*,  
20 (1999) Genet. Anal. 15, 179-187; Brink *et al.*, (2000) Theriogenology 53, 139-148; Colman, (1999) Genet. Anal. 15, 167-173; Eyestone, (1999) Theriogenology 51, 509-517; Baguisi *et al.*, (1999) Nat. Biotechnol. 17, 456-461; Prather *et al.*, (1999) Theriogenology 51, 487-498; Pain *et al.*, (1999) Cells Tissues Organs 165, 212-219; Fernandez *et al.*, (1999) Indian J. Exp. Biol. 37, 1085-1092; U.S. Patent Nos. 5,908,969; 5,792,902;  
25 5,892,070; 6,025,540).

The practice of the present invention will employ the conventional terms and techniques of molecular biology, pharmacology, immunology and biochemistry that are within the ordinary skill of those in the art. For example, see Sambrook *et al.*, (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press.

30 Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize



the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

5

## EXAMPLES

### Example 1 - Recombinant proteins, fusion proteins and protein tags

Cyclin E, p27, SKP1, and SKP2 were each cloned into pVL1392 (PharMingen) vector as glutathione-S-transferase (GST) fusion proteins. In addition to GST fusion  
10 proteins, SKP2 or SKP2-like proteins could be fused with a protein interaction domain such as Max, which binds to c-Myc, to target c-Myc for ubiquitination and degradation in cells. Human CUL-1, SKP2 and SKP1 cDNA were also cloned directly into baculovirus pVL1392 or pVL1393 expression vectors. The construction of these baculoviruses was accomplished as previously described (Zhang *et al.*, (1995) Cell 82, 915-925). The  
15 baculoviruses for CDK2 and GST-cyclin A were also constructed as previously described (Zhang *et al.*, (1995) Cell 82, 915-925). The cDNA clone encoding human E1 ubiquitin was cloned into the baculovirus expression vector, pAcSG-His-NT (PharMingen), as a histidine<sub>6</sub> tagged protein. In addition, SKP2 or SKP2-like proteins could be tagged with a protein interaction domain such as Max, which binds to c-Myc, to target c-Myc for  
20 ubiquitination and degradation in cells.

The E1 protein was expressed in the baculovirus expression system and purified by ubiquitin affinity chromatography (Yu *et al.*, (1998) Proc. Natl. Acad. Sci. USA 95, 11324-11329). The purification was monitored by protein staining and the E1 activity was assayed by covalent conjugation of biotinylated ubiquitin (Pagano *et al.*, (1995) Science  
25 269, 682-685). For <sup>35</sup>S- labeled p27, SF9 cells were infected with baculoviruses encoding GST-p27. Forty hours post-infection, cells were labeled with <sup>35</sup>S-methionine for three hours as described (Zhang *et al.*, (1995) Cell 82, 915-925). The labeled GST-p27 protein was isolated by glutathione Sepharose beads and the p27 portion was released from the beads by thrombin treatment for thirty minutes at room temperature (Calbiochem) (Guan  
30 *et al.*, (1991) Anal. Biochem. 192, 262-267). Thrombin was subsequently inactivated by

one mM phenylmethyl-sulfonyl fluoride (PMSF). The purified p27 is monitored by autoradiography and quantified by protein staining and Western-blot analysis.

To assemble cyclin E/CDK2, GST-cyclin E and CDK2 baculoviruses were individually expressed in SF9 cells. The lysates were prepared in hypotonic buffer (20 mM Hepes, pH 7.2, 5 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.5 mM DTT). The lysates containing GST-cyclin E and CDK2 were mixed and incubated in the presence of 10 mM ATP at 30 °C for one hour to assemble the active cyclin E/CDK2 kinase. The kinase was then affinity purified using the glutathione beads and quantified by protein staining and Western blot. The activity of purified kinase was monitored by the histone H1 assay (Zhang *et al.*, (1995) Cell 82, 915-925). To produce SCF<sup>SKP2</sup> complex, baculoviruses encoding GST-SKP1 and CUL-1, in the presence or absence of baculoviruses encoding SKP2, were co-infected into insect SF9 cells and were affinity purified using a glutathione Sepharose column. The successful assembly of the complex was monitored and quantified by protein staining and Western-blot analysis (Figure 15). The *in vitro* translated proteins were produced and labeled with <sup>35</sup>S-methionine in TNT rabbit reticulocyte lysates according to the manufacturer's instructions (Promega).

The human CDC34 cDNA clone was cloned into pGEXKG as a GST fusion protein and expressed in bacteria BL21. GST-CDC34 was isolated by glutathione column and the GST portion was removed by thrombin. The CDC34 protein was further purified with a MonoQ column and monitored by protein staining. The methyl ubiquitin and ubiquitin aldehyde were commercially obtained (BostonBiochem).

Anti-p27 (sc-528) antibodies were purchased commercially (Santa Cruz Biotechnology). Rabbit anti-cyclin E, SKP2, and CDK2 polyclonal antibodies and anti-HA epitope tag monoclonal antibody (12CA5) were described previously (Zhang *et al.*, (1995) Cell 82, 915-925; Xiong *et al.*, (1993) Nature 366, 701-714). For some experiments, a monoclonal anti-human cyclin E antibody (HE12) and a polyclonal anti-mouse cyclin E antibody (M20) were used (Santa Cruz). The anti-T7-tag monoclonal antibody was obtained from Novagen. Immunoprecipitation and Western-blot analyses were performed as described previously (Zhang *et al.*, (1995) Cell 82, 915-925). For direct Western-blotting, cells were lysed directly in 0.1% SDS, and viscosity was reduced by passing the lysates through a 22-gauge needle. Approximately 40 µg of proteins were

loaded directly onto an SDS-polyacrylamide gel for Western-blot analysis. Identical results were obtained from direct Western-blot analyses as from immunoprecipitation followed by Western-blot analyses.

5     **Example 2 - Phosphorylation-dependent p27 degradation**

Selective p27 degradation in cell free systems has been reported previously in synchronized S-phase extracts but not in G1 cell extracts (Nguyen *et al.*, (1999) Mol. Cell. Biol. 19, 1190-1201; Brandeis & Hunt (1995) EMBO J. 15, 5280-5289). To determine the proteins that control p27 stability, cytosolic extracts from asynchronized and exponentially  
10     growing HeLa cells were prepared.

HeLa cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum at 37°C. For extract preparation, suspension HeLa cells were grown to  $0.5-1 \times 10^6$  cells/ml (log-phase) and extracts were prepared as previously described (Brandeis & Hunt (1996) EMBO J. 15, 5280-5289). Cell pellets  
15     were washed twice with phosphate-buffered saline (PBS) and then with hypotonic buffer. The cells were re-suspended in two volumes of hypotonic buffer. They were lysed by Dounce homogenization using a loose pestle. The cytosolic extracts were prepared by centrifugation at 15,000 rpm using a Sorvall SS34 rotor. Aliquots of the extracts were immediately frozen in liquid nitrogen and stored at -80°C.

For a typical degradation reaction of p27, 200 µg cytosolic extract was used in a total volume of 50 µl, with no greater than 20% dilution of the extract. The reaction mixture also contained 2 mM ATP, 20 mM creatine phosphate, 50 µg/ml creatine kinase, 20 mM Hepes, pH7.2, 1 mM DTT, and 10 mM MgCl<sub>2</sub>. The reactions were initiated by adding <sup>35</sup>S-labeled p27 (0.25-0.5 µg) and cyclin E/CDK2 (1-3 µg) and incubation at 30°C  
25     for one to three hours. The amount of cyclin E/CDK2 required for p27 degradation was titrated batchwise for different extract preparations to determine the necessary threshold level of cyclin E/CDK2. The requirement of cyclin E/CDK2 is also dependent on the amount of exogenously added p27, reflecting the fact that p27 serves both as an inhibitor and a substrate for the kinase. Substantial amounts of endogenous p27 were present in the  
30     extract which was also degraded by addition of cyclin E/CDK2. The reactions were stopped by adding 0.1% SDS, followed by one ml of lysis buffer and in the presence of

protease inhibitors (5 µg/ml of leupeptide, soybean trypsin inhibitor and aprotinin plus 100 mM benzamidine). The reaction products were immunoprecipitated using p27 antibodies, fractionated in SDS-PAGE, and visualized by autoradiography. Degradation of endogenous p27 in the extracts was monitored by directly loading onto an SDS-PAGE in the SDS sample buffer, followed by Western-blotting with p27 antibodies.

Recombinant <sup>35</sup>S-labeled p27 was not degraded when incubated with this cytosolic extract indicating that p27 was quite stable. Addition of an active cyclin E/CDK2 kinase to the extract led to the rapid degradation of p27 (Figures 1A, 1B). The requirement of cyclin E/CDK2 for p27 destruction was due to its ability to phosphorylate p27 at threonine 187, which has been shown to trigger p27 degradation (Sheaff *et al.*, (1997) Genes Dev. 11, 1464-78; Vlach *et al.*, (1997) EMBO J. 16, 5334-44). Conversion of threonine 187 to glycine (T187G) stabilized p27 in the extract and confirmed the requirement for phosphorylation of threonine 187 (Figure 1A). In the presence of cyclin E/CDK2, a fraction of p27 slightly shifted its electrophoretic mobility which was sensitive to phosphatase (Figure 1C), indicating that these proteins are phosphorylated forms of p27.

A fraction of p27 was converted into multiple and high molecular weight species in the presence of cyclin E/CDK2 (Figure 1C), which are insensitive to phosphatase treatment. Addition of specific inhibitors of 26S proteasome, such as MG132, stabilized p27 and resulted in accumulation of both the phosphorylated and the high molecular weight forms of p27 (Figure 1C). Modified ubiquitins such as methyl ubiquitin and ubiquitin aldehyde also caused accumulation of p27 ladders (Figure 2) due to their ability to interfere with the degradation rate or inhibition of deubiquitination of highly polyubiquitinated proteins (Hershko *et al.*, (1987) Proc. Natl. Acad. Sci. USA 84, 1829-1833). These observations indicate that the high molecular weight species of p27 are the poly-ubiquitinated. This *in vitro* system therefore faithfully recapitulated the ubiquitin-dependent p27 degradation in a cyclin E/CDK2-dependent process which requires phosphorylation of the threonine 187 residue on p27.

### Example 3 - Alteration of p27 levels by depletion of SKP1, SKP2 & CUL-1

Using the *in vitro* p27 degradation system, the potential involvement of candidate ubiquitin E3 ligases, the SCF complexes (SKP1, CDC53/Cullins, F-box proteins), for p27

degradation was examined. The SCF complexes represent a conserved family of protein complexes that target phosphorylated proteins for ubiquitin-dependent proteolysis (Patton *et al.*, (1998) Trends Genet. 14, 236-243; Maniatis, (1999) Gene Dev. 13, 505-510).

CUL-1 was examined first to determine whether a human CDC53 homologue is necessary for p27 degradation.

For depletion of CUL-1, SKP1 or SKP2 proteins from HeLa extracts, four mg of affinity purified CUL-1, SKP1 or SKP2 antibodies (Zhang *et al.*, (1995) Cell 82, 915-925) or IgG were coupled to one ml protein A-Sepharose column. Five to ten ml of HeLa extracts were used to pass through the antibody-protein A column three times at 4 °C. The flow-through fractions from the columns were collected and examined for the efficiency of depletion using Western blot analysis. These fractions were then used as depleted extracts. For restoration of p27 degradation activity in SKP2 depleted extract, two µg of purified SKP2, SCF<sup>SKP2</sup> or SC (no SKP2) complexes were added to SKP2 depleted extracts and the degradation of p27 was monitored as described above.

Depletion of CUL-1 abolished the ability of the extracts to degrade p27 while parallel mock depletion using purified IgG from the pre-immune serum had no effect (Figure 3A). Western-blot analysis confirmed that CUL-1 was removed from the extracts by the column (Figure 2D). Specific immuno-depletion of another component of the SCF complex, SKP1, also resulted in the inhibition of p27 degradation which led to an increase in p27 levels (Figures 3B, 3C).

The requirement for CUL-1 and SKP1 for p27 degradation implies that an F-box protein is involved. The F-box protein is a component in the SCF complexes that interacts directly with the phosphorylated substrates and thus defines the substrate specificity for ubiquitination (Maniatis, (1999) Genes Dev. 13, 505-510; Skowyra *et al.*, (1997) Cell 91, 209-219; Winston *et al.*, (1999) Genes Dev. 13, 270-83). To identify the F-box protein(s) that specifically bind to p27, <sup>35</sup>S-labeled HeLa cell extracts were incubated with GST-p27 either with or without prior phosphorylation by cyclin E/CDK2. Examination of the labeled proteins specifically associated with the phosphorylated GST-p27 beads revealed the presence of a 45 kDa protein, which is similar to the molecular weight of the F-box protein SKP2.

To determine whether SKP2 is involved in p27 degradation, HeLa extracts were subjected to immuno-depletion with an affinity purified SKP2 antibody column. Removal of SKP2 by immunodepletion of SKP2 from the extract resulted in the inhibition of p27 degradation activity in the extract (Figure 3C, 3D). This data in combination with the SKP1 depletion experiments indicates that depletion of SKP proteins results in modulation of SKP activity which can increase expression of p27.

#### Example 4 - SKP2 binds to phosphorylated p27

To directly examine the specific binding of SKP2 to the phosphorylated form of p27, a pair of peptides corresponding to the carboxy-terminal end of p27 (amino acids 175-198) was synthesized. Threonine 187 was phosphorylated in the first peptide but not in the second peptide (Figure 4A). The peptides were each coupled to SulfoLink agarose beads which were then used as affinity resins for binding analysis of F-box proteins. These peptides were initially tested to determine if they could interact with several known F-box proteins, including SKP2,  $\beta$ -TrCP and MD6, as well as a number of unpublished F-box proteins identified through EST database search. *in vitro* translated and  $^{35}\text{S}$ -labeled F-box proteins were incubated with the p27 peptide beads. Analysis of the F-box proteins associated with p27 peptide beads revealed a specific interaction between SKP2 and the phosphorylated threonine 187 p27 peptide (Figure 4B). No significant interactions were observed if the non-phosphorylated form of the peptide was used. Specific associations between the p27 phosphopeptide and other available F-box proteins were not detected (Figure 4B, data not shown). These data indicate that SKP2 can interact selectively and specifically with the p27 phosphopeptide.

To determine whether endogenous SKP2 in the HeLa extract can also interact with p27 phosphopeptide, the peptide beads were incubated with the extracts. The peptides, containing either the carboxy-terminal end of p27 (amino acids 175-198), CSDGSPNAGSVEQTPKKPGLRRRQT, and phosphopeptides CSDGSPNAGSVEQ\*TPKKPGLRRRQT (\*T denotes phosphorylated threonine 187 of p27) (SEQ ID NO: 1) were synthesized using the peptide synthesis facility at the Yale University School of Medicine. The phosphorylated threonine 187 (\*T) and the non-phosphorylated forms of the p27 carboxy-terminal peptides were conjugated to

SulfoLink beads (Pierce) through the cysteine residue added at the amino-terminus of the peptides according to manufacturer's instruction (Pierce). For coupling reactions, 0.5 mg of peptides were conjugated onto two ml of Sulfolink beads for thirty minutes and the residue sites on the beads was blocked by 20 mM cysteine for two hours at room

5 temperature. The beads were washed extensively first with PBS followed by hypotonic buffer and stored at 4 °C. For F-box protein binding assays, 10 µl of the *in vitro* translated F-box proteins, including SKP2, β-TrCP, and MD6, were mixed 20 µl peptide beads in 250 µl of lysis buffer containing protease inhibitors (5 µg/ml leupeptide, trypsin inhibitor, aprotinin, and 100 mM benzamidine) and 100 mM NaF. Binding assays were performed  
10 at 4 °C for one hour with agitation. The beads were washed with detergent buffer for four times and the proteins associated with the beads were analyzed. A similar procedure was used for the extract binding except 100-400 µg of HeLa extracts were used as the source of SCF complexes, replacing the *in vitro* translated F-box proteins.

Western-blot analysis detected a strong and specific interaction between  
15 endogenous SKP2 and the p27 phosphopeptide (Figure 4C). The p27 peptide without threonine 187 phosphorylation did not significantly interact with SKP2. As a control for SKP2 binding, binding of β-TrCP to the p27 peptides was also measured. β-TrCP is an F-box protein that binds and targets phosphorylated β-catenin and IB for ubiquitin-dependent degradation (Maniatis, (1999) Genes Dev. 13, 505-510). The data  
20 indicated that although β-TrCP was also present in the extract, no interactions between β-TrCP and the p27 phosphopeptide were detected in these assays (Figure 4C). These studies confirm that endogenous SKP2 in the HeLa extract specifically recognizes and binds to the phosphorylated form of p27. In addition to SKP2 binding only to the phosphorylated form p27 peptide, it also binds only to the phosphorylated form of cyclin E  
25 peptide SPLPSGLL\*TPPQSGKKQSSGPEMA (amino acids 372-395 where \*T denotes phosphorylated threonine 187 of p27) (SEQ ID NO: 4). SKP2 can therefore be inhibited by a phosphopeptide other than p27 phosphopeptide.

Previous studies indicated that SKP2 interacts with SKP1 and CUL-1 *in vivo* (Yu  
30 *et al.*, (1998) Proc. Natl. Acad. Sci. USA 95, 11324-11329; Lisztwan *et al.*, (1998) EMBO J. 17, 368-83; Lyapina *et al.*, (1998) Proc. Natl. Acad. Sci. USA 95, 7451-7456; Michel *et al.*, (1998) Cell Growth Differ. 9, 435-449). SKP2 interactions with SKP1 and CUL-1

were also observed in the HeLa extracts (Figure 4D). It was necessary to confirm that SKP1 and CUL-1 could interact directly with the p27 since depletion of either of these proteins from the extracts also abolished p27 degradation. Confirmation of such a direct interaction would eliminate the possibility that other indirect mechanisms were responsible for the increase in p27 following removal of SKP1 or CUL-1. Using the peptide bead pull-down assays, specific interactions of SKP1 or CUL-1 with the phosphorylated threonine p27 peptide beads were detected in contrast to the non-phosphorylated peptide where no such interaction was detected (Figure 4C). To determine whether SKP2 mediates SKP1 and CUL-1 binding to p27 phosphopeptide, SKP1 or CUL-1 binding in SKP2 depleted extracts was examined. Depletion of SKP2 from the extract significantly reduced the binding of SKP1 (Figure 4E) or CUL-1 (data not shown) to the p27 phosphopeptide beads, although the total levels of SKP1 and CUL-1 in the extract were not substantially altered by SKP2 depletion (Figure 4E and data not shown). These studies indicate that SKP2 is the SCF component that binds to the phosphorylated threonine 187 of p27. Upon SKP2 binding to phosphorylated p27, SKP2 associates with SKP1 and CUL-1 and targets p27 for ubiquitin-dependent degradation by the 26S proteasome. Modulation of the activity of SKP2 can therefore increase the levels of p27.

#### Example 5 - SKP2-dependent degradation of p27

The effect of addition of the SCF<sup>SKP2</sup> complex to the SKP2 depleted extract was investigated to determine if restoration of SKP2 is sufficient to restore p27 degradation activity. Recombinant SCF<sup>SKP2</sup> complexes were expressed, assembled using the baculovirus expression system and purified. When the recombinant SCF<sup>SKP2</sup> complex was added back into the SKP2 depleted extract, restoration of p27 degradation was observed (Figure 5A). Restoration of p27 degradation was dependent on the presence of SKP2 in the complex because complexes assembled in the absence of SKP2 could not rescue the SKP2 deficiency in the extract. Addition of purified SKP2 alone could partially rescue p27 degradation in the SKP2 depleted extract (data not shown) but the assembled SCF<sup>SKP2</sup> complex consistently produced better restoration, indicating that the SCF<sup>SKP2</sup> complex itself is required for p27 degradation. When SKP2 expression is under the control of a tetracycline-inducible promotor in HeLa cells, removal of tetracycline results in expression



of SKP2. Induction of SKP2 resulted in approximately a significant decrease in total cellular p27. Since SKP2 only targets the Thr187-phosphorylated p27 for degradation, the down-regulation of p27 by expressing SKP2 indicates that SKP2 is rate limiting where sufficient CUL-1 and SKP-2 are present.

5

#### Example 6 - SKP2-dependent ubiquitination of p27

p27 ubiquitination was also assayed directly using the recombinant SCF<sup>SKP2</sup> complex. In a purified system containing the recombinant SCF<sup>SKP2</sup> complex, cyclin E/CLK2, ubiquitin activation enzyme E1, and ATP, a fraction of p27 was converted into multiple high molecular weight species (Figure 5B). The formation of high molecular weight p27 was dependent on the presence of ubiquitin and CDC34, a conserved E2 conjugating enzyme that is implicated in SCF-mediated ubiquitination (King *et al.*, (1996) Science 274, 1652-1659; Plon *et al.*, (1993) Proc. Natl. Acad. Sci. USA 90, 10484-10488). The E2 conjugating enzyme for SCF<sup>SKP2</sup> is probably a human CDC34 homolog. These data suggest that the SCF<sup>SKP2</sup> complex can ubiquitinate p27 in the presence of E1 and E2. However, p27 ubiquitination using the purified proteins was not very efficient. It is possible that SCF<sup>SKP2</sup> may require additional modifications or activities for efficient p27 ubiquitination.

#### 20 Example 7 - SKP2 binds to phosphorylated cyclin E

N-acetyl-L-leucyl-L-leucinal-L-norleucinal (LLNL) and hydroxyurea (HU) were purchased from Sigma. The cyclin E carboxy peptides (residues 371-394) CASPLPSGLLTTPQSGKKQSSGPEM containing either the Thr380-phosphorylated (TP-CP) or non-phosphorylated (TP-C) forms were synthesized and coupled to Sulfo-Link agarose beads (Pierce) as described previously (Tsvetkov *et al.*, (1999) Curr. Biol. 9, 661-664) (TP: corresponding to Thr380 and Pro381 in cyclin E). Cyclin E mutant peptides, TA-CP (CASPLPSGLLTAPQSGKKQSSGPEM) (SEQ ID NO: 5), SP-C and SP-CP (CASPLPSGLLTSPQSGKKQSSGPEM) (SEQ ID NO: 6), were synthesized accordingly. A cysteine residue was added to the amino-terminal end of these peptides to facilitate coupling to the beads. The cyclin E cDNA was tagged by the T7-epitope tag at its amino-terminus in pCGT, and its expression was under CMV promoter control. The SKP2

dominant negative mutant (SKP2DN) lacking the F-box was constructed as described (Carrano *et al.*, (1999) Nat. Cell. Biol. 1, 193-199). Both the wild-type and mutant SKP2 were cloned into the retrovirus vector pBabe. The full-length cDNA clones of FBL-2, -5, -6 and -8 were commercially purchased (Research Genetics) and were sequenced for confirmation.

To identify the F-box protein(s) that might bind to the phosphorylated Thr380 in cyclin E, a pair of peptides that correspond to the carboxy-terminal end of cyclin E that includes the critical Thr380 (Figure 6A) were synthesized. One peptide (TP-CP) contained the phosphorylated Thr380, and the other had a nonphosphorylated Thr380 (TP-C). Each of these peptides was immobilized onto agarose beads, which were then used to determine the binding of various F-box proteins. The F-box proteins were *in vitro* translated and <sup>35</sup>S-methionine-labeled. They were used directly to test for binding to TP-CP or TP-C beads. Using this assay, it was determined that SKP2 selectively interacted with the cyclin E phosphopeptide TP-CP, while no detectable interactions were observed between SKP2 and the nonphosphorylated cyclin E peptide TP-C. The interaction between the phosphorylated cyclin E peptide and SKP2 is specific, since it was not possible to detect the binding of cyclin E TP-CP to other F-box proteins, including various FBLs (Winston *et al.*, (1999) Curr. Biol. 9, 1180-1182; Cenciarelli *et al.*, (1999) Curr. Biol. 9, 1177-9), which bears close homologies to SKP2 as well as the more distantly related  $\beta$ -TrCP and MD6 (Figure 6B and data not shown).

SKP2 normally forms a complex with SKP1 and CUL-1 (Tsvetkov *et al.*, (1999) Curr. Biol. 9, 661-664). To determine whether the SCF<sup>SKP2</sup> complex binds specifically to the phosphorylated cyclin E peptide, a cytosolic HeLa cell extract was used as the source of SCF complexes (Figure 6C). Thus, SKP2, SKP1, and CUL-1 all interact specifically with the cyclin E phosphopeptide TP-CP but not with the nonphosphorylated cognate peptide TP-C (Figure 6C). To rule out the possibility that SKP2 nonspecifically binds to phosphorylated peptides, a number of mutant peptide derivatives were synthesized in which either Thr380 in cyclin E was converted into serine or phosphoserine (SP-C or SP-CP) or Pro381 was converted into alanine but with Thr380 remaining phosphorylated (TA-CP). Binding assays indicate that SKP2 did not interact with the mutant SP-CP and TA-CP phosphopeptides. Similar results were obtained in a peptide competition experiment in

which increasing amounts of either TP-CP, TA-CP, or SP-CP phosphopeptides were used as competitors for the association between SKP2 and the TP-CP beads (Figure 6D).

#### Example 8 - Phosphorylation-dependent cyclin E degradation

5 SKP2 expression is periodic in a cell-cycle-dependent manner, with a peak level in the S phase (Zhang *et al.*, (1995) Cell 82, 915-925). Recent evidence suggests that SKP2 is a limiting component of the SCF<sup>SKP2</sup> complex for S phase entry and for the degradation of p27 (Sutterluty *et al.*, (1999) Nat. Cell Biol. 1, 207-214; Tsvetkov *et al.*, (1999) Curr. Biol. 9, 661-664; Carrano *et al.*, (1999) Nat. Cell. Biol. 1, 193-199; Zhang *et al.*, (1995) 10 Cell 82, 915-925). To determine whether cyclin E is a target for ubiquitination by SKP2, the levels of T7-epitope-tagged cyclin E were examined after its transfection into HeLa cells in the presence or absence of SKP2. SKP2 expression caused a substantial reduction in the levels of co-expressed cyclin E. This effect is dependent on the Thr380 residue in cyclin E. When Thr380 was converted into glycine (T380G), which could not be 15 phosphorylated, the mutant cyclin E was much more resistant to SKP2 (Figure 7A). Pulse-and-chase experiments indicated that SKP2 significantly shortened the half-life of the cyclin E protein (Figure 8A).

In addition, expression of SKP2 induced the formation of high-molecular-weight ladders of cyclin E (Figure 7B-E) in both 293 and mouse embryonic fibroblast cells. The 20 SKP2-dependent formation of high-molecular-weight ladders of cyclin E was mostly abolished if Thr380 of cyclin E was mutated into glycine (T380G). To determine whether the high-molecular-weight species were polyubiquitinated forms of cyclin E, the effect of expressing an HA-tagged ubiquitin (HAUb) on cyclin E was examined. Expression of HAUb also led to the accumulation of the high-molecular-weight forms of cyclin E similar 25 to the ones induced by SKP2 (Figure 7C). Immunoprecipitation with anti-HA epitope antibody followed by Western-blotting with T7-tagged cyclin E revealed that the high-molecular-weight species of cyclin E were polyubiquitinated forms of cyclin E (Figure 7D).

Furthermore, expression of SKP2 greatly promoted high levels of incorporation of 30 HAUb into cyclin E, as compared with that of HAUb alone (Figure 7D). These observations indicate that expression of SKP2 is sufficient to cause the polyubiquitination

of cyclin E *in vivo*. In addition, the effect of T380G mutation in cyclin E on the polyubiquitination of cyclin E indicates that ubiquitination is dependent on the presence of Thr380. However, in the absence of Thr380, a weaker but detectable level of cyclin E ubiquitination was observed (Figure 7B). This ubiquitination was also promoted by SKP2. Although the phosphorylated Thr380 provides a major binding site for SKP2, there exists additional minor sites in cyclin E that can be used for SKP2 binding and cyclin E ubiquitination.

#### Example 9 - SKP2-dependent ubiquitination of cyclin E independent of p27

The F-box proteins usually interact directly with their phosphorylated substrates. To determine the potential association of SKP2 with full-length cyclin E, T7-tagged wild-type or the T380G mutant form of cyclin E was expressed in the presence or in the absence of N-acetyl-L-leuciny-L-leucinal-L-norleucinal (LLNL), a specific inhibitor of the 26S proteasome *in vivo* (Figure 8B). Immunoprecipitation followed by Western-blotting indicated that both un-ubiquitinated and ubiquitinated forms of cyclin E were associated with SKP2 (Figure 8B). The cyclin E T380G mutant was also found to be associated with SKP2 (Fig. 3B) but to a lesser extent. This is consistent with the earlier observation (Figure 7B) that the phosphorylated Thr380 is a major site for SKP2 binding but that there are additional minor sites in cyclin E for SKP2 binding and ubiquitination. Since cyclin E-SKP2 interaction (Figure 8B) and ubiquitination (Figure 7E) occurred in p27<sup>-/-</sup> mouse embryonic cells, this indicates that cyclin E ubiquitination (Figure 7E and 8B) and its interaction with SKP2 (Figure 8B) are independent of p27.

Although cyclin E ubiquitination is independent of p27, in the presence of co-expressed CDK inhibitor p27, cyclin E degradation was inhibited even in the presence of SKP2 (Figure 8C). This observation indicates that p27 might inhibit cyclin E autophosphorylation on Thr380, leading to resistance to SKP2-mediated ubiquitin-dependent degradation of cyclin E. The effect of p27 is not to be due to a competition between p27 and cyclin E for SKP2 binding, since a non-phosphorylated mutant form of p27 in which the critical Thr187 was converted into glycine (T187G) did not bind. This data is consistent with the previous report that p27 inhibits the Thr380-dependent cyclin E degradation (Clurman *et al.*, (1996) Genes Devel. 10, 1979-1990).

Expression of SKP2 also affects the endogenous cyclin E level. When SKP2 was expressed in cells using recombinant retrovirus delivery system, a significant decrease in endogenous cyclin E levels was observed (Figure 9A and B). As observed before, ectopic expression of SKP2 also led to the reduction of p27 levels. The possibility that cyclin E down-regulation is due to a secondary effect of SKP2 on the S phase was eliminated since SKP2 caused the decrease of cyclin E even in cells that were synchronized in the S phase by hydroxyurea (Figure 7B). Conversely, expression of a dominant-negative SKP2 (DN) that is defective in F-box, a binding site for SKP1, caused the accumulation of endogenous cyclin E (Figure 8C and D). Such an effect on the endogenous cyclin E is independent of p27, since SKP2DN-mediated elevation of cyclin E could occur in p27<sup>-/-</sup> mouse embryonic fibroblasts (Figure 8D). This observation is consistent with our finding that SKP2-mediated-ubiquitination of cyclin E occurs in p27<sup>-/-</sup> mouse embryonic fibroblasts (Figure 7E). This data indicates that SKP2-mediated cyclin E ubiquitination is p27-independent.

Applicants have identified SKP2 as an F-box protein that mediates ubiquitin-dependent degradation of cyclin E. SKP2 is an F-box protein that is expressed in late G1, S, and G2 phases, playing a role in S phase of the cell cycle (Zhang *et al.*, (1995) Cell 82, 915-925). SCF<sup>SKP2</sup> binds and targets the CDK inhibitor p27 for ubiquitin-dependent degradation. In addition, SKP2 also interacts with cyclin E and plays a role in the ubiquitin-dependent degradation of cyclin E. This SKP2-mediated cyclin E ubiquitination and degradation is mostly dependent on the presence of Thr380 in cyclin E (Figure 14), although weak cyclin E ubiquitination in the absence of Thr380 was also promoted by SKP2 *in vivo*.

Applicants have also identified that SKP2 performs a dual function during the G1/S transition. It is required for the ubiquitin-dependent degradation of p27 in late G1 (Sutterluty *et al.*, (1999) Nat. Cell. Biol. 1, 207-14; Tsvetkov *et al.*, (1999) Curr. Biol. 9, 661-664; Carrano *et al.*, (1999) Nat. Cell. Biol. 1, 193-199). The degradation of p27 by SCF<sup>SKP2</sup> activates cyclin E/CDK2 and promotes entry into the S-phase (Sutterluty *et al.*, (1999) Nat. Cell. Biol. 1, 207-14; Coats *et al.*, (1996) Science 272, 877-880). Once cells are in the S phase, cyclin E is degraded which may be required for terminating the S-phase initiation events, allowing the cells to progress from the S phase into the G2 phase

(Clurman *et al.*, (1996) Genes Dev. 10, 1979-1990; Won *et al.*, (1996) EMBO J. 15, 4182-4193).

Applicants have determined that a number of phosphorylation dependent and ubiquitin-dependent degradation events occur during the G1/S transition, which are temporally regulated. The expression of SKP2 in the late G1 and S phases leads to assembly of the SCF<sup>SKP2</sup> complex. Previous reports suggest that the phosphorylation status of p27 and cyclin E could be temporally separated. p27 phosphorylation on the critical Thr187 has been shown to occur in the late G1 phase and p27 ubiquitination has been reported to require its binding to the cyclin E/CDK2 complex (Montagnoli *et al.*, (1999) Genes Dev. 13, 1181-1189). The phosphorylation of Thr187 in p27 triggers the binding of SKP2, leading to the subsequent ubiquitin-dependent degradation of p27.

It has been shown that binding of p27 to cyclin E/CDK complexes inhibits the activity of cyclin E/CDK2 and cyclin E degradation (Clurman *et al.*, (1996) Genes Dev. 10, 1979-1990). The binding of p27 therefore prevents phosphorylation on Thr380 in cyclin E or there is a competition between p27 and cyclin E for the binding of SKP2. p27 binding can also cause a conformational change in cyclin E so that Thr380 in cyclin E is not exposed for phosphorylation or SKP2 binding. Applicants have determined that SKP2 binds to the p27 phosphopeptide with higher affinity than that of cyclin E peptide (data not shown). Thus the affinities between SKP2 and p27 or cyclin E may also affect the ubiquitination rate of p27 and cyclin E by SKP2. Once p27 is degraded, the cyclin E/CDK2 kinase activity is activated, leading to the S-phase entry. Activation of cyclin E also leads to its autophosphorylation in Thr380 (Clurman *et al.*, (1996) Genes Dev. 10, 1979-1990; Won & Reed, (1996) EMBO J. 15, 4182-4193). The phosphorylation of Thr380 promotes the SKP2 binding which in turn results in the ubiquitin-dependent degradation of cyclin E.

The efficiency of the ubiquitination reaction by the SCF complexes is very high. Based on the *in vitro* and *in vivo* p27 and cyclin E degradation using SKP2, the reaction efficiency can be 80-90% or even higher to 100% (Figure 14). This is a low estimation, since SKP2 only binds to phosphorylated substrates, the complete reaction is thus dependent on the extent of the substrate phosphorylation, which in turn relies on activities of kinases and phosphatase that regulate the levels of substrate phosphorylation *in vivo* or

in the cell extracts. Conversely, using the p27 phosphopeptide, it is possible to deplete almost all SKP2 in the cell extract. This indicates that SKP2 can bind to its substrates with very high affinity.

5     **Example 10 - SKP2 Fusion Proteins Capable of Altering Substrate Specificity**

          The substrate-specificity of SCF complexes can be altered if the substrate-binding domains of the F-box protein such as LRR in SKP2 or WD repeats in  $\beta$ -TRCP are replaced by other protein-protein interaction motifs. As a first test for such a possibility, a hybrid protein that contains the amino-terminus of  $\beta$ -TRCP up to its F-box motif was  
10     created (residues 1-204, the F-box is located between residues 148-191). However, the substrate-targeting domain of the WD repeats is replaced by the LRR region of SKP2 (residues 169-435, the F-box is between residues 112-151) (Figure 10). Such a fusion creates a hybrid protein ( $\beta$ -TRCP.N/SKP2.C) that contains the F-box region of  $\beta$ -TRCP and the SKP2 substrate-binding domain (leucine-rich repeats or LRR). This hybrid  $\beta$ -  
15     TRCP.N/SKP2.C protein would be expected to have an altered substrate specificity. Instead of normally targeting  $\beta$ -catenin and I $\kappa$ B by  $\beta$ -TRCP, the hybrid protein should target SKP2-specific substrates, such as cyclin E or p27, for ubiquitination and degradation. As expected, when this fusion protein is introduced into 293 cells, it is fully active to ubiquitinate cyclin E for polyubiquitination in the same way as SKP2 (Figure  
20     11). Thus, swapping the domain of F-box proteins can alter the substrate specificity of F-box proteins.

          By fusing the amino-terminus of  $\beta$ -TRCP and the carboxy terminus of SKP2, a fully active hybrid  $\beta$ -TRCP.N/SKP2.C protein was produced to target cyclin E for ubiquitination. The results from the  $\beta$ -TRCP.N/SKP2.C hybrid protein suggest that  
25     alteration of specificity of the F-box proteins can be made. However, it could not be ruled out that the LRR region of SKP2 contains a motif that is also required for SKP1 binding or the SCF ubiquitination activity.

          Sequence comparison (Figure 13) has revealed the presence of a relatively conserved motif at the carboxy-terminal region of SKP2 (residues 321-374) and  $\beta$ -TRCP  
30     (residues 429-497). This motif is also present in the yeast F-box CDC4 carboxy-terminus (residues 388-463). For convenience this domain will be designated SCM for SKP2 C-

terminal motif. The role of this motif is to mediate the interaction between the F-box proteins and SKP1 or other components of the SCF complexes. This possibility is based on our finding that von Hippel-Lindau disease protein ("VHL"), a human tumor suppressor protein that binds to a SKP1-like protein, elongin C/SIII in a putative SCF-like ubiquitin E3 ligase CUL-2/elongin B/C complex, also has this domain (residues 146-195) (Stebbins *et al.*, (1999) Science 284, 455-461). Fusing the F-box, a protein interaction domain, such as Max or the MDM2 amino-terminus, and this conserved SCM domain, should improve the ubiquitination of targeted protein by various hybrid proteins.

The VHL binds to CUL-2 and Elongin C (also called SIIIC) (Pause *et al.*, (1997) Proc. Natl. Acad. Sci. USA 94, 2156-2161). Human CUL-2 is a close homologue of CUL-1 while Elongin C/SIIIC shares substantial homology with SKP1 (Pause *et al.*, (1997) Proc. Natl. Acad. Sci. USA 94, 2156-2161; Kipreos *et al.*, (1996) Cell 85, 829-839). The formation of the VHL/CUL-2-Elongin C complex (Duan *et al.*, (1995) Science 269, 1402-1406; Kibel *et al.*, (1995) Science 269, 1444-1446), with additional components such as Elongin B (also called SIIIB, a ubiquitin-like protein) and Rbx1 (Kamura *et al.*, (1999) Science 284, 657-661; Duan *et al.*, (1995) Science 269, 1402-1406; Kibel *et al.*, (1995) Science 269, 1444-1446), has been suggested to contain an SCF-like ubiquitin ligase activity (Pause *et al.*, (1997) Proc. Natl. Acad. Sci. USA 94, 2156-2161). Immuno-purified SCF complexes from both yeast (Seol *et al.*, (1999) Genes Dev. 13, 1614-1626) and human cells can ubiquitinate proteins associated with SCF complexes if they are co-incubated with ubiquitin, ATP, CDC34 E2 conjugating enzyme and E1 (Figure 14). Similar ubiquitination activity has been found to associate with the purified VHL/CUL-2/Elongin C/Elongin B complex (Lisztwan *et al.*, (1999) Genes Dev. 13, 1822-1833). Since it was determined that VHL shares certain homology with the SCM of SKP2 in the  $\alpha$ -domain, it is likely VHL/CUL-2/Elongin C/Elongin B is an SCF-like E3 ubiquitin ligase that uses VHL as a substrate targeting subunit. Under such circumstances, the protein-knockout technique proposed for SKP2 or other F-box proteins can also be applied to the use of VHL. Thus it is expected that if one fuses a protein interaction domain with VHL, the VHL fusion protein should act to ubiquitinate the target protein through the interaction between the protein interaction domain and the target.



CUL-1 and CUL-2 belong to the cullin family (Kipreos *et al.*, (1996) Cell 85, 829-839), which so far contains several additional members such as CUL-3 (Singer *et al.*, (1999) Genes Dev. 13, 2375-2387; Michel & Xiong, (1998) Cell Growth Differ. 9, 435-449), CUL-4A and 4B (Kipreos *et al.*, (1996) Cell 85, 829-839; Chen *et al.*, (1998) Cancer Res. 58, 3677-3683), vasopressin-activated calcium-mobilizing receptor-1 (Stankovic *et al.*, (1997) Genomics 40, 267-276), and anaphase-promoting complex 2 (APC2) (Stankovic *et al.*, (1997) Genomics 40, 267-276). Based on the homology between CUL-1 and other members of cullin family, it is expected that these cullin family members should act as ubiquitin E3 ligases. In addition, if similar fusion proteins for the substrate-targeting components of these cullin family members are constructed, it is possible to alter the substrate specificity of these ubiquitin E3 ligases in the same design as proposed for that of SCF complexes.

Example 11 - F-box antagonist peptides block the SKP1/F-box protein interaction

The F-box region is a peptide motif composed of 40-50 amino acids that is present in a variety of otherwise unrelated proteins (Winston *et al.*, (1999) Curr. Biol. 9, 1180-1182; Cenciarelli *et al.*, (1999) Curr. Biol. 9, 1177-1179). The F-box region is required for the SKP1 interaction for the assembly of the SCF complex (SKP1, CUL-1, F-box proteins) (Zhang *et al.*, (1995) Cell 82, 915-925; Bai *et al.*, (1996) Cell 86, 263-274). Since F-box proteins regulate many important proteins such as  $\beta$ -catenin, I $\kappa$ B, p27, cyclin E that are involved in tumorigenesis, signal transduction, cell cycle regulation, and development (Maniatis, (1999) Genes Dev. 13, 505-510; Koepp *et al.*, (1999) Cell 97, 431-434; Sidow *et al.*, (1999) Nat. Genet. 23, 104-107; Kawakami *et al.*, (2000) Curr. Biol. 10, 463-466), it is anticipated that modulation of the various SCF complexes would provide a means to control and alter the biological consequences that involve the SCF activity. One way to interfere the SCF activity to alter the developmental, cell cycle, tumorigenic, or signaling pathways is to use the peptides or peptide analogues derived from the F-box region and use them as an antagonist peptide for SCF activities.

The method can be used for targeted protein knockout for genetic and biochemical analysis in cells and animals. It will help to elucidate the normal functions of a target protein in cells and animal or in human by creating deficient mutants of targeted protein.

It can also be used to correct the diseases by altering the level of the disease protein or its antagonists. It can be used for testing the function and regulation of the targeted protein in diseases, drug sensitivity, development, cell growth and differentiation, programmed cell death, behavior, gene expression patterns, and learning and memory.

- 5           The method can also be used for detecting protein-protein or peptide-protein interaction by fusing SKP2 or F-box proteins with a protein or peptide that bind to a target protein. Ubiquitination of the target protein can be used as the means of detection.

#### Example 12 - SKP2-Like Proteins

- 10           SKP2-like proteins are proteins that contain a SKP1 interacting domain that is homologous to the SKP2 sequence LPDELLLGIFSCCLPELLKVSGVCKRWYRL ASDESLWQTLDL (SEQ ID NO: 2) (amino acids 112-154) (Zhang *et al.*, (1995) Cell 82, 915-925; Bai *et al.*, (1996) Cell 86, 263-274; Patton *et al.*, (1998) Trends Genet. 14, 236-243; Skowyra *et al.*, (1997) Cell 91, 209-219; Yu *et al.*, (1998) Proc Natl Acad Sci USA.  
15           95, 11324-11329; Winston *et al.*, (1999) Genes Dev. 13, 270-283; Winston *et al.*, (1999) Curr. Biol. 9, 1180-1182; Cenciarelli *et al.*, (1999) Curr. Biol. 9, 1177-1179). The SKP1 interacting domain is the region on the SKP2 protein that interacts with the SKP1 protein. This region is also called the F-box for SKP1 binding (Bai *et al.*, (1996) Cell 86, 263-274).

- The SKP1 interacting domain is present in a variety of proteins from yeast to human,  
20           including: (1) *Xenopus* b-TrCP which has the sequence LPARGLDHIAENILSYLDAKSL CSAELVCKEWYRV TSDGMLWKKL (SEQ ID NO: 3) (amino acids 135-157); (2) human b-TrCP (amino acids 148-192), which is identical to SEQ ID NO: 3 (Bai *et al.*, (1996) Cell 86, 263-274; Winston *et al.*, (1999) Genes Dev. 13, 270-283; Spevak *et al.*, (1993) Mol. Cell. Biol. 13, 4953-4966); and (3) some yeast proteins such as CDC4 and  
25           GRR1 (Bai *et al.*, (1996) Cell 86, 263-274; Skowyra *et al.*, (1997) Cell 91, 209-219).

- These proteins can replace SKP2 to form a complex with SKP1 and CUL-1 or their yeast homologues SKP1 or CDC53. Like SKP2, they bind to phosphorylated proteins and target them for ubiquitination and degradation. More than ten human SKP2-like proteins have been identified and obtained through ESTdatabase (Figure 12) (Winston *et al.*,  
30           (1999) Curr. Biol. 9, 1180-1182; Cenciarelli *et al.*, (1999) Curr. Biol. 9, 1177-1179. See also, for example, SEQ ID NO: 26-61.

Although the present invention has been described in detail with reference to examples above, it is understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims. All cited patents and publications referred to in this application are

5    herein incorporated by reference in their entirety.

We claim,

1. A method of altering the level of polypeptide in a cell comprising altering the amount of one or more of the proteins selected from the group consisting of SKP1, SKP2,  
5 SKP2-like protein and CUL-1.
2. The method of claim 1 wherein the polypeptide is phosphorylated.
3. The method of claim 1 wherein the SKP-2 like protein is selected from the  
10 group consisting of ZF1 (SEQ ID NO: 27), ZF3 (SEQ ID NO: 29), ZF4 (SEQ ID NO: 31), ZF5 (SEQ ID NO: 33), ZF6 (SEQ ID NO: 35), ZF7 (SEQ ID NO: 37), ZF8 (SEQ ID NO: 39), ZF9 (SEQ ID NO: 41), ZF11 (SEQ ID NO: 43), ZF13 (SEQ ID NO: 45), ZF16 (SEQ ID NO: 47), ZF18 (SEQ ID NO: 49), ZF19 (SEQ ID NO: 51), ZF20 (SEQ ID NO: 53), ZF23 (SEQ ID NO: 55), ZF24 (SEQ ID NO: 57), ZF25 (SEQ ID NO: 59) and ZF26 (SEQ  
15 ID NO: 61).
4. The method of claim 1 wherein the polypeptide is p27 (SEQ ID NO: 65).
5. The method of claim 1 wherein the polypeptide is selected from the group  
20 consisting of cyclin E (SEQ ID NO: 63), Max (SEQ ID NO: 9), Mad (SEQ ID NO: 11), c-Myc (SEQ ID NO: 13), MDM2 (SEQ ID NO: 15), p53 (SEQ ID NO: 17), Bax (SEQ ID NO: 19), Bad (SEQ ID NO: 21) and Bcl-2 (SEQ ID NO: 23).
6. The method of claim 1 wherein the level of polypeptide is increased by  
25 decreasing the amount of SKP2.
7. The method of claim 1 wherein the level of polypeptide is reduced by increasing the amount of SKP2.
- 30 8. A method of altering the level of SKP2 comprising altering the amount of p27 polypeptide which is available for binding with SKP2.

9. A method of modulating the activity of SKP2 comprising contacting SKP2 with a peptide comprising a SKP2 interaction domain which is available for binding with SKP2.

5

10. The method of claim 9 wherein the peptide is phosphorylated.

11. The method of claim 10 wherein the SKP2 interaction domain is derived from p27.

10

12. The method of claim 10 wherein the SKP2 interaction domain is derived from cyclin E.

13. The method of claim 9 wherein the peptide comprises any one of the amino acid sequences of SEQ ID NO: 1, 2, 3, 4, 5, or 6.

15

14. A method of treating a tumor in a mammal comprising altering the level of SKP protein in the cells of said tumor.

15. The method of claim 14 wherein the SKP protein is SKP2 or allelic variants thereof.

20

16. A method of detecting a tumor in a mammal wherein the level of SKP2 is used as a diagnostic indicator to determine the progression of said tumor.

25

17. A method of detecting a tumor in a mammal wherein the level of SKP2 is used as a prognostic indicator to determine the progression of said tumor.

18. A method of monitoring the treatment of a tumor in a mammal wherein the level of SKP2 is used as a diagnostic indicator to monitor the success of a said treatment.

30

19. A method of monitoring the treatment of a tumor in a mammal wherein the level of SKP2 is used as a prognostic indicator to monitor the success of a said treatment.

20. A method of testing an agent for the ability to modulate an interaction between SKP2 and a target protein wherein the method comprises:

- (a) fusing SKP2 with a target protein interaction domain to produce a SKP2 fusion protein;
- (b) contacting the agent, the SKP2 fusion protein and the target protein; and
- (c) determining whether the interaction of the SKP2 fusion protein with the target protein has been modulated by the agent.

21. A method of altering the level of a target protein in a cell comprising inserting a heterologous target protein interaction domain into SKP2 or a SKP2-like protein to produce a fusion protein, and contacting the fusion protein with the target protein.

15

22. The method of claim 21 wherein the SKP-2 like protein is selected from the group consisting of ZF1 (SEQ ID NO: 27), ZF3 (SEQ ID NO: 29), ZF4 (SEQ ID NO: 31), ZF5 (SEQ ID NO: 33), ZF6 (SEQ ID NO: 35), ZF7 (SEQ ID NO: 37), ZF8 (SEQ ID NO: 39), ZF9 (SEQ ID NO: 41), ZF11 (SEQ ID NO: 43), ZF13 (SEQ ID NO: 45), ZF16 (SEQ ID NO: 47), ZF18 (SEQ ID NO: 49), ZF19 (SEQ ID NO: 51), ZF20 (SEQ ID NO: 53), ZF23 (SEQ ID NO: 55), ZF24 (SEQ ID NO: 57), ZF25 (SEQ ID NO: 59) and ZF26 (SEQ ID NO: 61).

20

23. A method of altering the level of a target protein in a cell comprising expressing a cDNA coding for a SKP2 fusion protein comprising a SKP2 protein fused with a target protein interaction domain which is specific for the target protein.

25

24. A method of ubiquitinating a target protein in a cell comprising fusing a target protein interaction domain with SKP2, and permitting the SKP2 fusion protein to contact with the target protein.

30

25. The method of either claim 23 or 24 wherein the target protein is selected from the group consisting of p27 (SEQ ID NO: 65), cyclin E (SEQ ID NO: 63), Max (SEQ ID NO: 9), Mad (SEQ ID NO: 11), c-Myc (SEQ ID NO: 13), MDM2 (SEQ ID NO: 15), p53 (SEQ ID NO: 17), Bax (SEQ ID NO: 19), Bad (SEQ ID NO: 21) and Bcl-2 (SEQ ID NO: 23).

26. A method of modulating protein ubiquitination in a cell comprising altering the amount of SKP2 which is available to facilitate protein ubiquitination.

27. A fusion protein comprising a first protein comprising at least one SKP2 C-terminal motif (SCM) capable of interacting with SKP1 and forming a complex with CUL-1 and a second protein which is capable of interacting with a heterologous target protein.

28. The fusion protein of claim 27 wherein the fusion protein contains only one SCM capable of interacting with SKP1.

29. The fusion protein of claim 27 wherein the SCM is selected from any one of the following proteins selected from the group consisting of SKP2 (SEQ ID NO: 67), ZF1 (SEQ ID NO: 27), ZF3 (SEQ ID NO: 29), ZF4 (SEQ ID NO: 31), ZF5 (SEQ ID NO: 33), ZF6 (SEQ ID NO: 35), ZF7 (SEQ ID NO: 37), ZF8 (SEQ ID NO: 39), ZF9 (SEQ ID NO: 41), ZF11 (SEQ ID NO: 43), ZF13 (SEQ ID NO: 45), ZF16 (SEQ ID NO: 47), ZF18 (SEQ ID NO: 49), ZF19 (SEQ ID NO: 51), ZF20 (SEQ ID NO: 53), ZF23 (SEQ ID NO: 55), ZF24 (SEQ ID NO: 57), ZF25 (SEQ ID NO: 59) and ZF26 (SEQ ID NO: 61).

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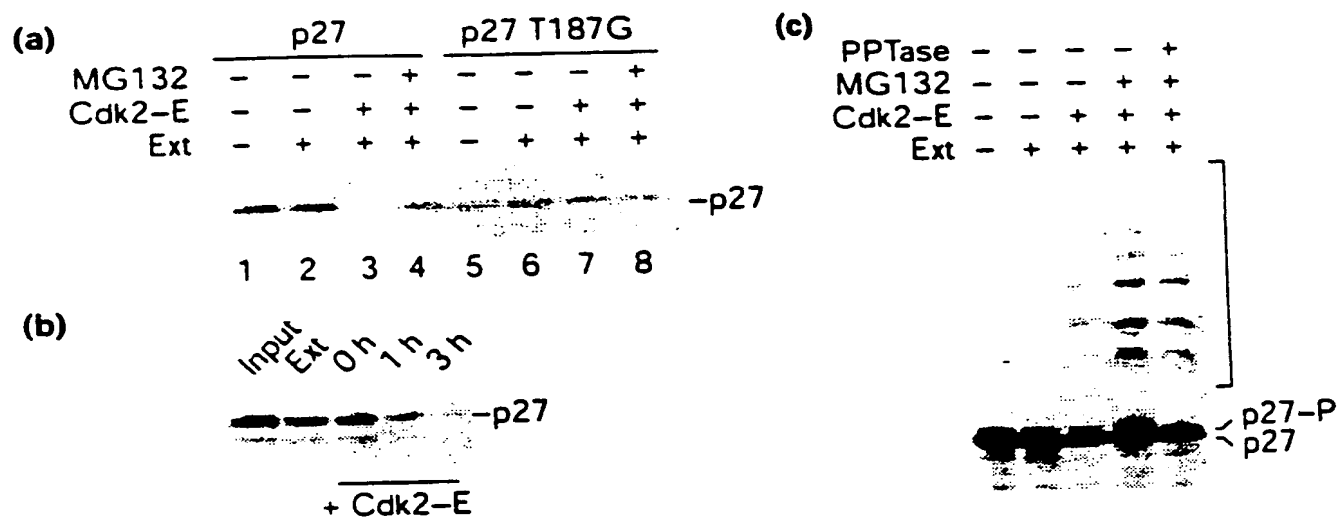
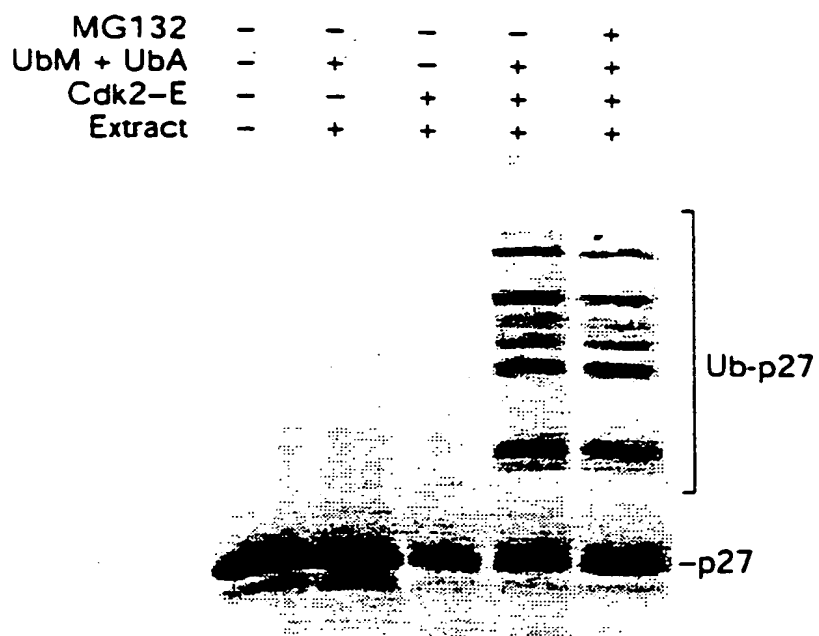


Figure 1



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**Figure 2**

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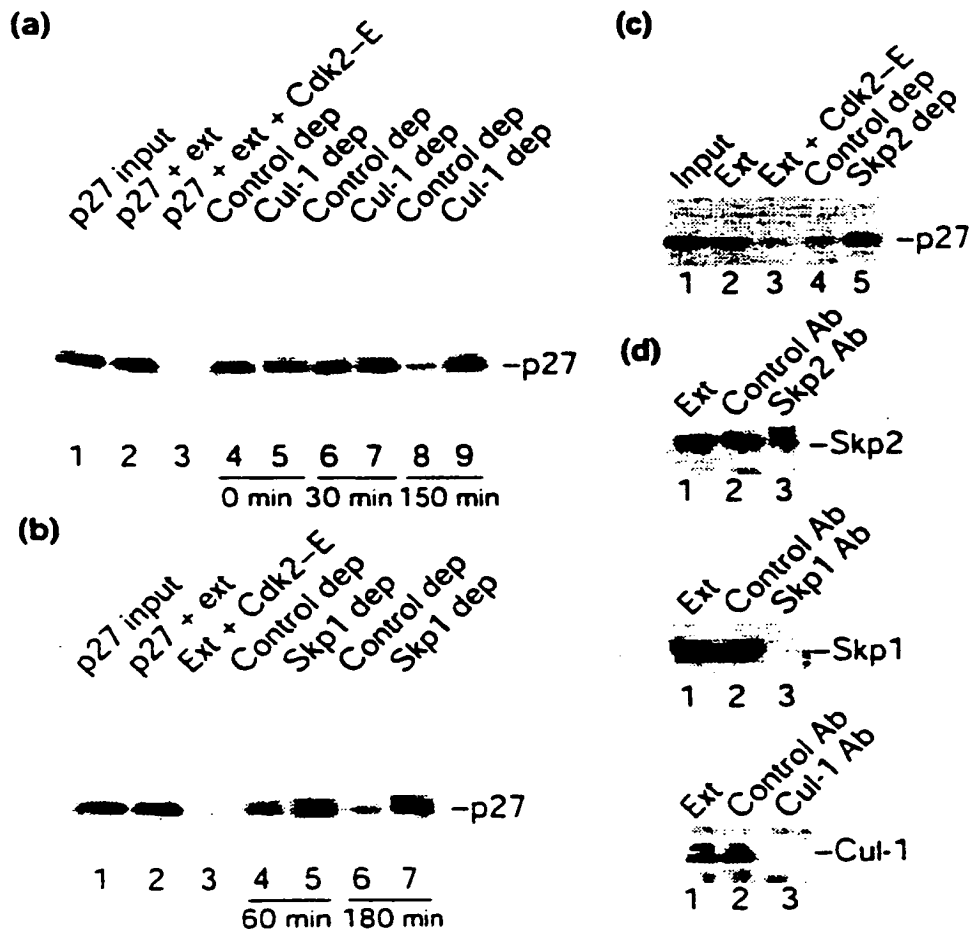


Figure 3

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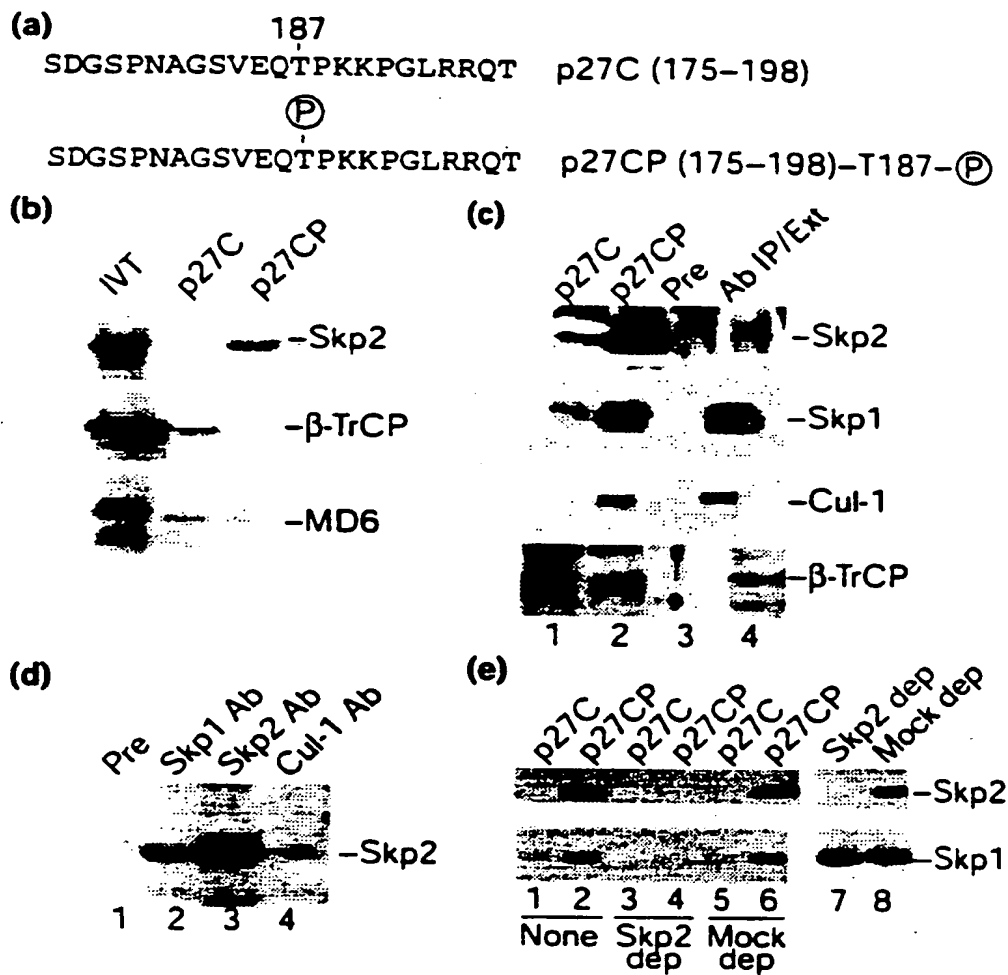


Figure 4

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(a)



(b)



Figure 5

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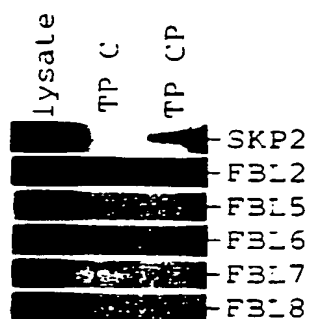
A.

Cyclin E peptides:

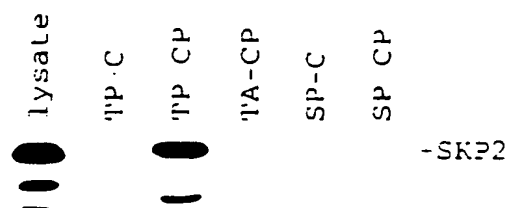
ASPLPSGLLTTPPQSGKKQSSGPEM TP-C (a.a. 371-394)

ASPLPSGLLT<sup>Ⓟ</sup>TPPQSGKKQSSGPEM TP-CP

B.



D.



C.

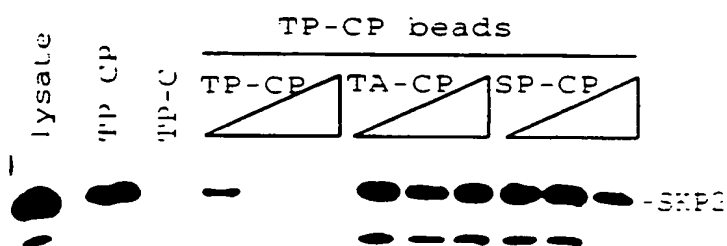
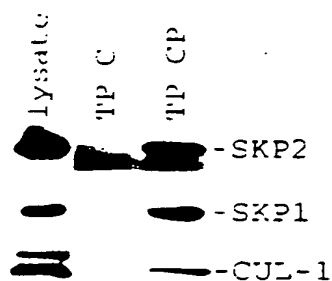


Figure 6

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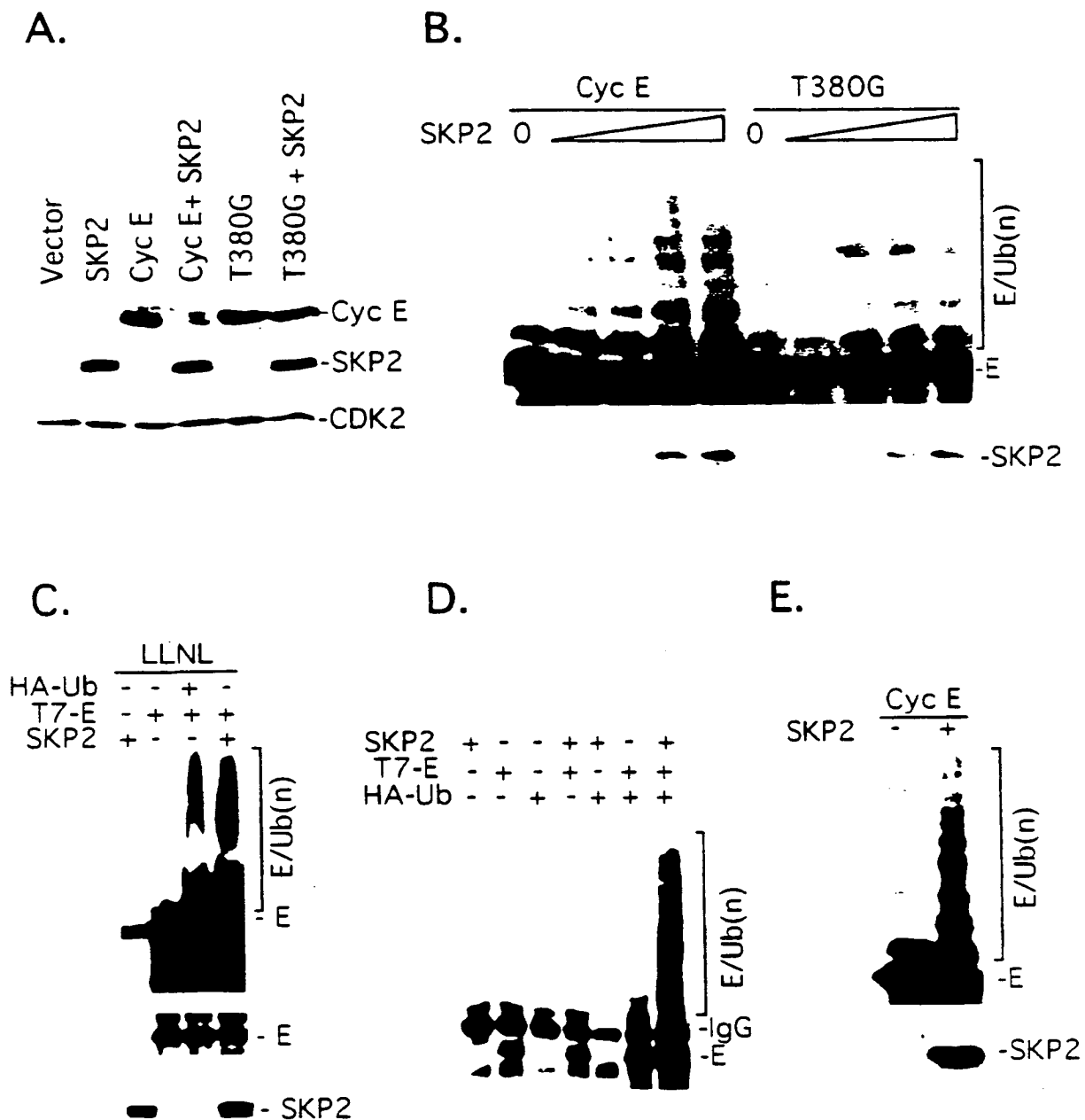
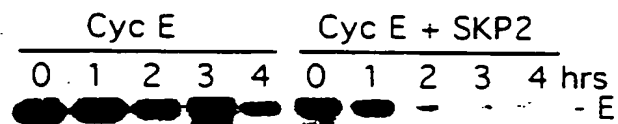


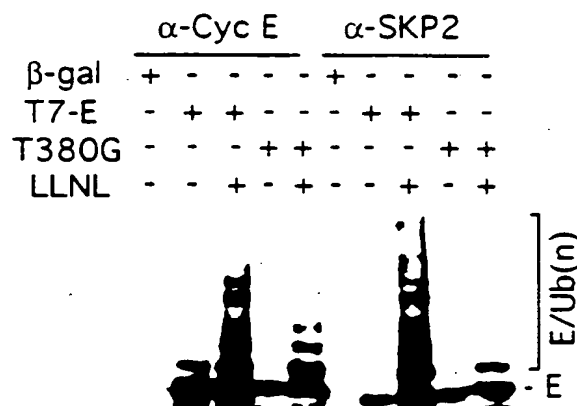
Figure 7

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A.



B.



C.

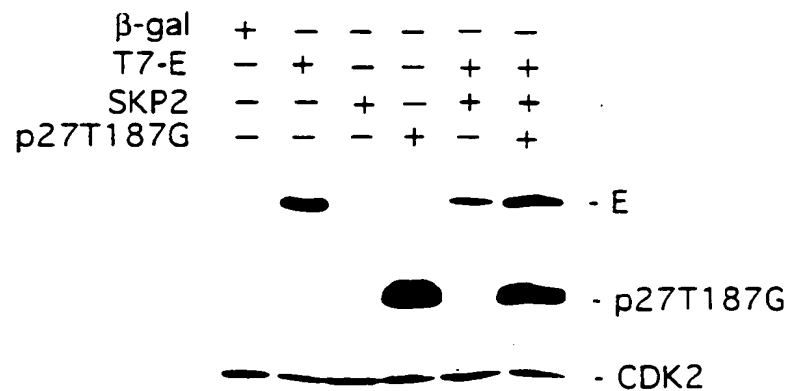
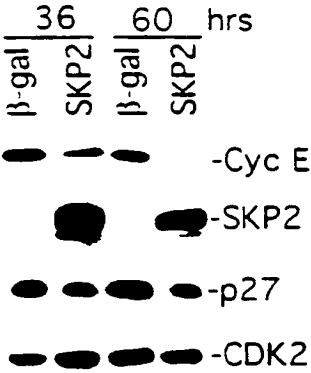


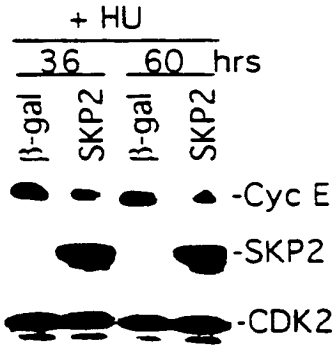
Figure 8

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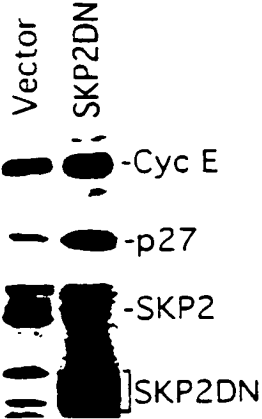
A.



B.



C.



D.

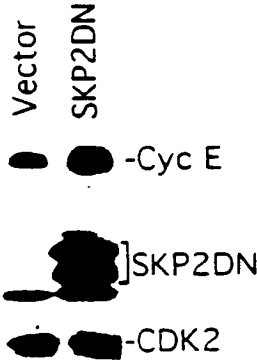
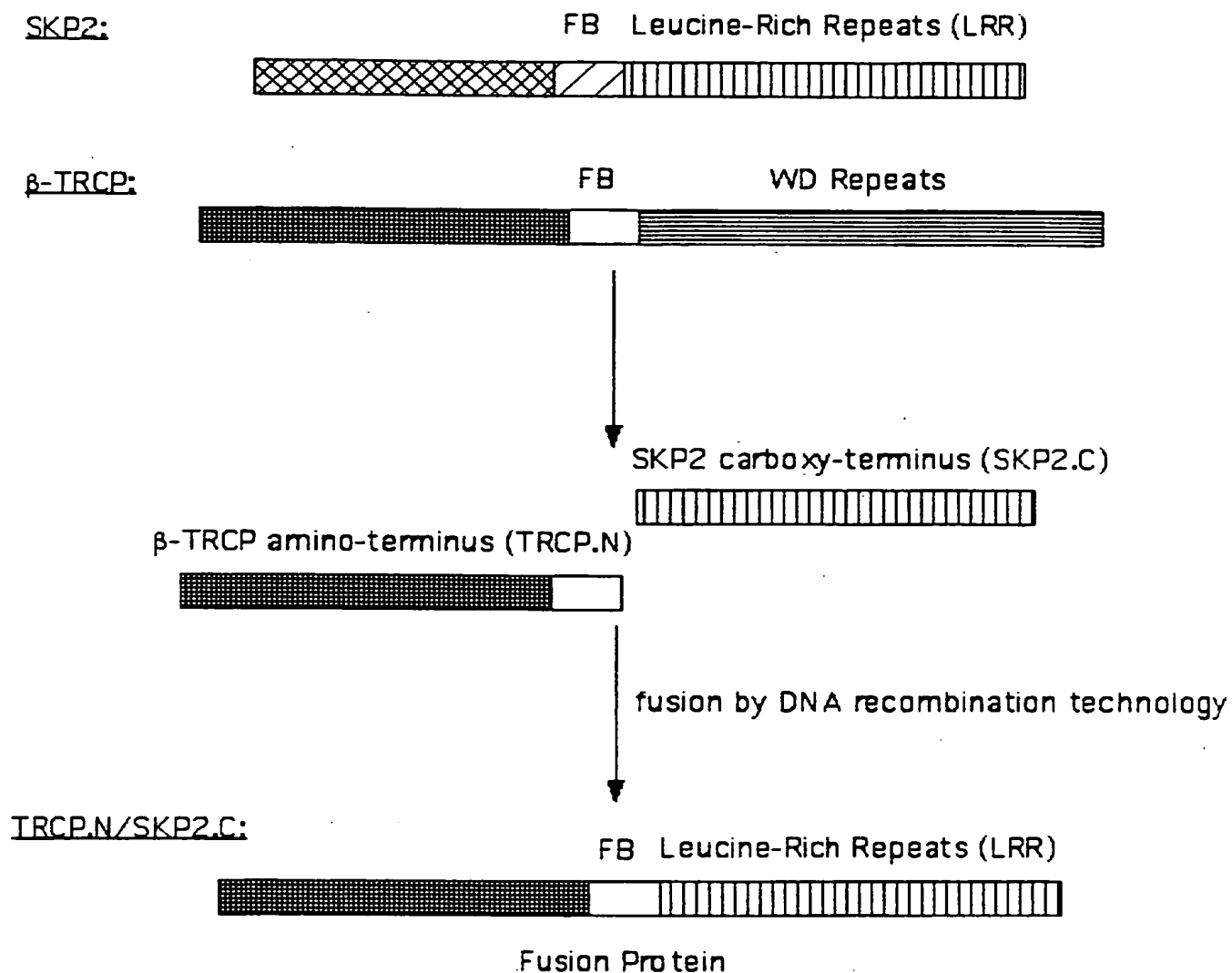


Figure 9



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Creating a hybrid fusion protein (TRCP.N/SKP2.C) consisting of the amino-terminal region of  $\beta$ -TRCP (a.a. 1-204) and the carboxy-terminal region of SKP2 (a.a. 169-435):

**Figure 10**

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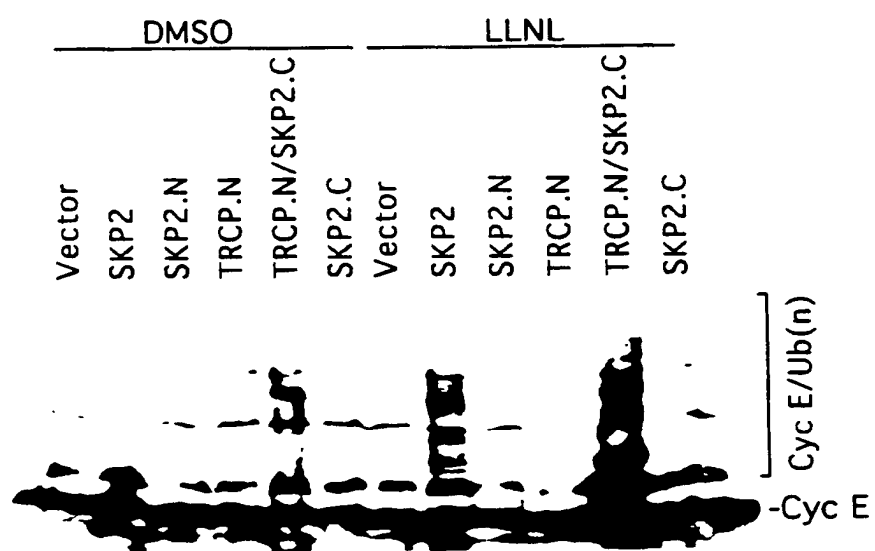


Figure 11

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Figure 12

C. Homologies between SKP2 and other F-box proteins in the F-box region:

	1	15	16	30	31	45	46	60	61	67	
1 SKP2	LPD	-----ELLGGIFS	CLCLPE	-----LLKV	SGVCKRWY-RLASDE	SLWQTLDL	-----	-----	-----	-----	43
2 ZF25	LPD	-----SVLLEIFS	YLPVRD	-----RIRI	SRVCHRWK-RLVDDR	WLWRHVDLT	-----	-----	-----	-----	44
3 ZF16	IPL	-----EILVQIFG	LLVAADGMPFLGRA	ARVCRRWQ-EAASQP	ALWHTVTTL	-----	-----	-----	-----	-----	48
4 ZF24	LPD	-----HSMVQIFS	FLPTNQ	-----LCRC	ARVCRRWY-NLAWDP	RLWRTIRLT	-----	-----	-----	-----	44
5 ZF8	LPI	-----DVQLYILS	FLSPHD	-----LCQL	GSTNHYWN-ETVRDP	ILWRYFLLR	-----	-----	-----	-----	44
6 ZF20	LPI	-----DVQLYILS	FLSPHD	-----LCQL	GSTNHYWN-ETVRDP	ILWRYFLLRD	-----	-----	-----	-----	45
7 ZF5	LPR	-----VLSVYIFS	FLDPRS	-----LCRC	AQVSWYWK-SLAELD	QLWMLKCL	-----	-----	-----	-----	43
8 ZF7	LPY	-----ELAINIFQ	YLDRKE	-----LGRC	AQVSKTWK-VIAEDE	VLWYRLCQQ	-----	-----	-----	-----	44
9 ZF1	LPK	-----ELLRIFS	FLDIVT	-----LCRC	AQISKAWN-ILALDG	SNWQRID	-----	-----	-----	-----	42
10 ZF3	LPP	-----EVMLSIFS	YLNPOE	-----LCRC	SQVSMKWS-QLTKTG	SLWKHLYPVHWARGD	WYSGPAT	-----	-----	-----	57
11 ZF4	LPD	-----EVVLKIFS	YLLEQD	-----LCRA	ACVCKRFS-ELANDP	ILWLGEVAHA	-----	-----	-----	-----	45
12 ZF9	LPP	-----ELSFTILS	YLNATD	-----LCLA	SCVWQ	-----DLANDE	LLWQGLCK	-----	-----	-----	40
13 ZF11	LPARGLDHIAENILS	YLDKAS	-----LCAA	ELVCKEWY-RVTSDG	MLWKKLIE	-----	-----	-----	-----	-----	47
14 ZF13	LPS	-----VPMEILS	YLDAYS	-----LLQA	AQVKNKNW-ELASSD	VLWRKLC	-----	-----	-----	-----	42
15 ZF23	LPT	-----DPLLLILS	FLDYRD	-----LINC	CYVSRRLS-QLSSHD	PLWRRHCKKYWLISE	EE	-----	-----	-----	52
16 ZF19	LPLLP	DSLVIYQIFL	SLGPAD	-----VLAA	GLVCRWQ-AVSRDE	FLWKEQFYR	-----	-----	-----	-----	47
17 ZF18	LLQ	-----DIILQVFK	YLPPLD	-----RAHA	SQVCRNWN-QVFHMP	DLWRCFEFE	-----	-----	-----	-----	44
18 ZF26	LPE	-----VLLLHMCS	YLDMRA	-----LGR	L	RRQIAWASLNSG	-----	-----	-----	-----	49
19 ZF6	LPL	-----ELSFYLLK	WLDPQT	-----LLTC	CLVSKQRNKVISACT	EVWQTACKNLGWQID	DSV	-----	-----	-----	54

A. Homology between the  $\alpha$  domain of the von Hippel-Linda protein (VHL) and the SCM domain of SKP2:

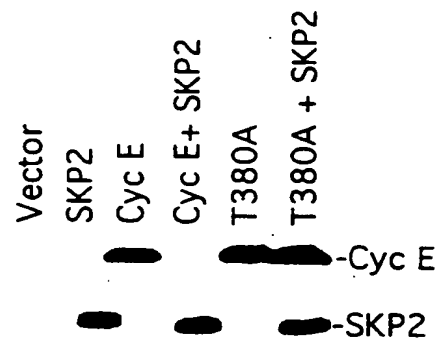
VHL: PIFANITLP-VYTLKERCLQVVRSLVKPEN---YRRLDIVRSLYEDLEDHPNVQ  
SKP2: PNLVHLDLSNSVMLKND~~CF~~QEFSQLNYLQHL~~SL~~SR~~CD~~YDIIPETLLELGEIPTLK

B. Homology between the SCM domain of  $\beta$ -TRCP, SKP2, and CDC4.Ca:

$\beta$ -TRCP: LRVLEGHEELVRCIR~~FD~~DNKRIVSGAYDGKIKVWDL  
SKP2: QLN~~YL~~QHL~~SL~~SR~~CD~~YDIIPETLLELGEIPTL~~KT~~LQV  
CDC4.Ca: THVFKGHNSTVRCLDIVEYKNIKYIVTGSRDNTLH

**Figure 13**

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**Figure 14**

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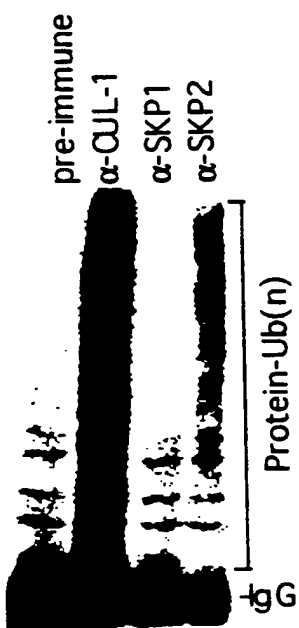


Figure 15

## SEQUENCE LISTING

<110> Zhang, Hui  
Tsvetkov, Lyuben  
Kondo, Takeshi

<120> Modulation of Protein Levels Using the SCF Complex

<130> 44574-5047-WO

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<150> US 60/137,494

<151> 1999-06-04

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<213> Homo sapiens

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Glu	Leu	Leu	Lys	Val	Ser	Gly	Val	Cys	Lys	Arg	Trp	Tyr	Arg	Leu	Ala
			20				25						30		

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Leu Asp Ala Lys Ser Leu Cys Ser Ala Glu Leu Val Cys Lys Glu Trp  
20 25 30  
Tyr Arg Val Thr Ser Asp Gly Met Leu Trp Lys Lys Leu  
35 40 45

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Lys	Lys	Gln	Ser	Ser	Gly	Pro	Glu	Met
		20				25		

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Ser	Asp	Glu	Glu	Gln	Pro	Arg	Phe	Gln	Ser	Ala	Ala	Asp	Lys	Arg	Ala	
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cat	cat	aat	gca	ctg	gaa	cga	aaa	cgt	agg	gac	cac	atc	aaa	gac	agc	147
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Gln Asn Ala Leu Leu Glu Gln Gln Gly Glu Ser Glu Ser
      95              100

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tcccgtggct cgggcccccg gtgcaga atg gcg gcg gcg gtt cgg atg aac atc 174

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1

5

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Gln Met Leu Leu Glu Ala Ala Asp Tyr Leu Glu Arg Arg Glu Arg Glu

10

15

20

25

gct gaa cat ggt tat gcc tcc atg tta cca tac aat aac aag gac aga 270

Ala Glu His Gly Tyr Ala Ser Met Leu Pro Tyr Asn Asn Lys Asp Arg

30

35

40

gat gcc tta aaa cgg agg aac aaa tcc aaa aag aat aac agc agt agc 318

Asp Ala Leu Lys Arg Arg Asn Lys Ser Lys Lys Asn Asn Ser Ser Ser

45

50

55

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Arg Ser Thr His Asn Glu Met Glu Lys Asn Arg Arg Ala His Leu Arg

60

65

70

ttg tgc ctg gag aag ttg aag ggg ctg gtg cca ctg gga ccc gaa tca 414

Leu Cys Leu Glu Lys Leu Lys Gly Leu Val Pro Leu Gly Pro Glu Ser

75

80

85

agt cga cac act acg ttg agt tta tta aca aaa gcc aaa ttg cac ata 462

Ser Arg His Thr Thr Leu Ser Leu Leu Thr Lys Ala Lys Leu His Ile

90

95

100

105

aag aaa ctt gaa gat tgt gac aga aaa gcc gtt cac caa atc gac cag 510

Lys Lys Leu Glu Asp Cys Asp Arg Lys Ala Val His Gln Ile Asp Gln

110

115

120

ctt cag cga gag cag cga cac ctg aag agg cag ctg gag aag ctg ggc 558

Leu Gln Arg Glu Gln Arg His Leu Lys Arg Gln Leu Glu Lys Leu Gly

125

130

135

att gag agg atc cgg atg gac agc atc ggc tcc acc gtc tcc tcg gag 606

Ile Glu Arg Ile Arg Met Asp Ser Ile Gly Ser Thr Val Ser Ser Glu

140

145

150

cgc tcc gac tcc gac agg gaa gaa atc gac gtt gac gtg gag agc acg 654

Arg Ser Asp Ser Asp Arg Glu Glu Ile Asp Val Asp Val Glu Ser Thr

155

160

165

gac tat ctc aca ggt gat ctg gac tgg agc agc agc agt gtg agc gac 702

Asp Tyr Leu Thr Gly Asp Leu Asp Trp Ser Ser Ser Ser Val Ser Asp

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195

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Gly Leu Val Pro Leu Gly Pro Glu Ser Ser Arg His Thr Thr Leu Ser  
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Leu Leu Thr Lys Ala Lys Leu His Ile Lys Lys Leu Glu Asp Cys Asp  
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Arg Lys Ala Val His Gln Ile Asp Gln Leu Gln Arg Glu Gln Arg His  
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Glu Ile Asp Val Asp Val Glu Ser Thr Asp Tyr Leu Thr Gly Asp Leu  
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 acgcggggag gctattctgc ccatttgggg acacttcccc gccgctgcca ggaccgctt 480  
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 365 370 375

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 380 385 390 395

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aag ctc att tct gaa gag gac ttg ttg cgg aaa cga cga gaa cag ttg 1839  
 Lys Leu Ile Ser Glu Glu Asp Leu Leu Arg Lys Arg Arg Glu Gln Leu  
 415 420 425

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 Lys His Lys Leu Glu Gln Leu Arg Asn Ser Cys Ala  
 430 435

taaggaaaac gattccttct aacagaaatg tcctgagcaa tcacctatga acttgtttca 1945  
 aatgcatgat caaatgcaac ctcacaacct tggctgagtc ttgagactga aagatttagc 2005  
 cataatgtaa actgcctcaa attggacttt gggcataaaa gaactttttt atgcttacca 2065  
 tctttttttt ttctttaaca gatttgtatt taagaattgt ttttaaaaaa ttttaa 2121

&lt;210&gt; 13

&lt;211&gt; 439

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 13

Met Pro Leu Asn Val Ser Phe Thr Asn Arg Asn Tyr Asp Leu Asp Tyr  
 1 5 10 15

Asp Ser Val Gln Pro Tyr Phe Tyr Cys Asp Glu Glu Glu Asn Phe Tyr  
 20 25 30

Gln Gln Gln Gln Gln Ser Glu Leu Gln Pro Pro Ala Pro Ser Glu Asp  
 35 40 45

Ile Trp Lys Lys Phe Glu Leu Leu Pro Thr Pro Pro Leu Ser Pro Ser  
 50 55 60

Arg Arg Ser Gly Leu Cys Ser Pro Ser Tyr Val Ala Val Thr Pro Phe  
 65 70 75 80  
 Ser Leu Arg Gly Asp Asn Asp Gly Gly Gly Gly Ser Phe Ser Thr Ala  
 85 90 95  
 Asp Gln Leu Glu Met Val Thr Glu Leu Leu Gly Gly Asp Met Val Asn  
 100 105 110  
 Gln Ser Phe Ile Cys Asp Pro Asp Asp Glu Thr Phe Ile Lys Asn Ile  
 115 120 125  
 Ile Ile Gln Asp Cys Met Trp Ser Gly Phe Ser Ala Ala Ala Lys Leu  
 130 135 140  
 Val Ser Glu Lys Leu Ala Ser Tyr Gln Ala Ala Arg Lys Asp Ser Gly  
 145 150 155 160  
 Ser Pro Asn Pro Ala Arg Gly His Ser Val Cys Ser Thr Ser Ser Leu  
 165 170 175  
 Tyr Leu Gln Asp Leu Ser Ala Ala Ala Ser Glu Cys Ile Asp Pro Ser  
 180 185 190  
 Val Val Phe Pro Tyr Pro Leu Asn Asp Ser Ser Ser Pro Lys Ser Cys  
 195 200 205  
 Ala Ser Gln Asp Ser Ser Ala Phe Ser Pro Ser Ser Asp Ser Leu Leu  
 210 215 220  
 Ser Ser Thr Glu Ser Ser Pro Gln Gly Ser Pro Glu Pro Leu Val Leu  
 225 230 235 240  
 His Glu Glu Thr Pro Pro Thr Thr Ser Ser Asp Ser Glu Glu Glu Gln  
 245 250 255  
 Glu Asp Glu Glu Glu Ile Asp Val Val Ser Val Glu Lys Arg Gln Ala  
 260 265 270  
 Pro Gly Lys Arg Ser Glu Ser Gly Ser Pro Ser Ala Gly Gly His Ser  
 275 280 285  
 Lys Pro Pro His Ser Pro Leu Val Leu Lys Arg Cys His Val Ser Thr  
 290 295 300  
 His Gln His Asn Tyr Ala Ala Pro Pro Ser Thr Arg Lys Asp Tyr Pro  
 305 310 315 320  
 Ala Ala Lys Arg Val Lys Leu Asp Ser Val Arg Val Leu Arg Gln Ile  
 325 330 335  
 Ser Asn Asn Arg Lys Cys Thr Ser Pro Arg Ser Ser Asp Thr Glu Glu  
 340 345 350  
 Asn Val Lys Arg Arg Thr His Asn Val Leu Glu Arg Gln Arg Arg Asn  
 355 360 365  
 Glu Leu Lys Arg Ser Phe Phe Ala Leu Arg Asp Gln Ile Pro Glu Leu



370	375	380
Glu Asn Asn Glu Lys Ala Pro Lys Val Val Ile Leu Lys Lys Ala Thr		
385	390	395 400
Ala Tyr Ile Leu Ser Val Gln Ala Glu Glu Gln Lys Leu Ile Ser Glu		
	405	410 415
Glu Asp Leu Leu Arg Lys Arg Arg Glu Gln Leu Lys His Lys Leu Glu		
	420	425 430
Gln Leu Arg Asn Ser Cys Ala		
435		

<210> 14  
 <211> 1476  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> CDS  
 <222> (1)..(1473)  
 <223> Human MDM2

<400> 14	
atg tgc aat acc aac atg tct gta cct act gat ggt gct gta acc acc	48
Met Cys Asn Thr Asn Met Ser Val Pro Thr Asp Gly Ala Val Thr Thr	
1 5 10 15	
tca cag att cca gct tcg gaa caa gag acc ctg gtt aga cca aag cca	96
Ser Gln Ile Pro Ala Ser Glu Gln Glu Thr Leu Val Arg Pro Lys Pro	
20 25 30	
ttg ctt ttg aag tta tta aag tct gtt ggt gca caa aaa gac act tat	144
Leu Leu Leu Lys Leu Leu Lys Ser Val Gly Ala Gln Lys Asp Thr Tyr	
35 40 45	
act atg aaa gag gtt ctt ttt tat ctt ggc cag tat att atg act aaa	192
Thr Met Lys Glu Val Leu Phe Tyr Leu Gly Gln Tyr Ile Met Thr Lys	
50 55 60	
cga tta tat gat gag aag caa caa cat att gta tat tgt tca aat gat	240
Arg Leu Tyr Asp Glu Lys Gln Gln His Ile Val Tyr Cys Ser Asn Asp	
65 70 75 80	
ctt cta gga gat ttg ttt ggc gtg cca agc ttc tct gtg aaa gag cac	288
Leu Leu Gly Asp Leu Phe Gly Val Pro Ser Phe Ser Val Lys Glu His	
85 90 95	
agg aaa ata tat acc atg atc tac agg aac ttg gta gta gtc aat cag	336
Arg Lys Ile Tyr Thr Met Ile Tyr Arg Asn Leu Val Val Val Asn Gln	
100 105 110	
cag gaa tca tcg gac tca ggt aca tct gtg agt gag aac agg tgt cac	384
Gln Glu Ser Ser Asp Ser Gly Thr Ser Val Ser Glu Asn Arg Cys His	
115 120 125	

ctt gaa ggt ggg agt gat caa aag gac ctt gta caa gag ctt cag gaa 432  
 Leu Glu Gly Gly Ser Asp Gln Lys Asp Leu Val Gln Glu Leu Gln Glu  
 130 135 140

gag aaa cct tca tct tca cat ttg gtt tct aga cca tct acc tca tct 480  
 Glu Lys Pro Ser Ser Ser His Leu Val Ser Arg Pro Ser Thr Ser Ser  
 145 150 155 160

aga agg aga gca att agt gag aca gaa gaa aat tca gat gaa tta tct 528  
 Arg Arg Arg Ala Ile Ser Glu Thr Glu Glu Asn Ser Asp Glu Leu Ser  
 165 170 175

ggt gaa cga caa aga aaa cgc cac aaa tct gat agt att tcc ctt tcc 576  
 Gly Glu Arg Gln Arg Lys Arg His Lys Ser Asp Ser Ile Ser Leu Ser  
 180 185 190

ttt gat gaa agc ctg gct ctg tgt gta ata agg gag ata tgt tgt gaa 624  
 Phe Asp Glu Ser Leu Ala Leu Cys Val Ile Arg Glu Ile Cys Cys Glu  
 195 200 205

aga agc agt agc agt gaa tct aca ggg acg cca tcg aat ccg gat ctt 672  
 Arg Ser Ser Ser Ser Glu Ser Thr Gly Thr Pro Ser Asn Pro Asp Leu  
 210 215 220

gat gct ggt gta agt gaa cat tca ggt gat tgg ttg gat cag gat tca 720  
 Asp Ala Gly Val Ser Glu His Ser Gly Asp Trp Leu Asp Gln Asp Ser  
 225 230 235 240

gtt tca gat cag ttt agt gta gaa ttt gaa gtt gaa tct ctc gac tca 768  
 Val Ser Asp Gln Phe Ser Val Glu Phe Glu Val Glu Ser Leu Asp Ser  
 245 250 255

gaa gat tat agc ctt agt gaa gaa gga caa gaa ctc tca gat gaa gat 816  
 Glu Asp Tyr Ser Leu Ser Glu Glu Gly Gln Glu Leu Ser Asp Glu Asp  
 260 265 270

gat gag gta tat caa gtt act gtg tat cag gca ggg gag agt gat aca 864  
 Asp Glu Val Tyr Gln Val Thr Val Tyr Gln Ala Gly Glu Ser Asp Thr  
 275 280 285

gat tca ttt gaa gaa gat cct gaa att tcc tta gct gac tat tgg aaa 912  
 Asp Ser Phe Glu Glu Asp Pro Glu Ile Ser Leu Ala Asp Tyr Trp Lys  
 290 295 300

tgc act tca tgc aat gaa atg aat ccc ccc ctt cca tca cat tgc aac 960  
 Cys Thr Ser Cys Asn Glu Met Asn Pro Pro Leu Pro Ser His Cys Asn  
 305 310 315 320

aga tgt tgg gcc ctt cgt gag aat tgg ctt cct gaa gat aaa ggg aaa 1008  
 Arg Cys Trp Ala Leu Arg Glu Asn Trp Leu Pro Glu Asp Lys Gly Lys  
 325 330 335

gat aaa ggg gaa atc tct gag aaa gcc aaa ctg gaa aac tca aca caa 1056  
 Asp Lys Gly Glu Ile Ser Glu Lys Ala Lys Leu Glu Asn Ser Thr Gln  
 340 345 350

gct gaa gag ggc ttt gat gtt cct gat tgt aaa aaa act ata gtg aat 1104  
 Ala Glu Glu Gly Phe Asp Val Pro Asp Cys Lys Lys Thr Ile Val Asn

355	360	365	
gat tcc aga gag tca tgt gtt gag gaa aat gat gat aaa att aca caa			1152
Asp Ser Arg Glu Ser Cys Val Glu Glu Asn Asp Asp Lys Ile Thr Gln			
370	375	380	
gct tca caa tca caa gaa agt gaa gac tat tct cag cca tca act tct			1200
Ala Ser Gln Ser Gln Glu Ser Glu Asp Tyr Ser Gln Pro Ser Thr Ser			
385	390	395	400
agt agc att att tat agc agc caa gaa gat gtg aaa gag ttt gaa agg			1248
Ser Ser Ile Ile Tyr Ser Ser Gln Glu Asp Val Lys Glu Phe Glu Arg			
405	410	415	
gaa gaa acc caa gac aaa gaa gag agt gtg gaa tct agt ttg ccc ctt			1296
Glu Glu Thr Gln Asp Lys Glu Glu Ser Val Glu Ser Ser Leu Pro Leu			
420	425	430	
aat gcc att gaa cct tgt gtg att tgt caa ggt cga cct aaa aat ggt			1344
Asn Ala Ile Glu Pro Cys Val Ile Cys Gln Gly Arg Pro Lys Asn Gly			
435	440	445	
tgc att gtc cat ggc aaa aca gga cat ctt atg gcc tgc ttt aca tgt			1392
Cys Ile Val His Gly Lys Thr Gly His Leu Met Ala Cys Phe Thr Cys			
450	455	460	
gca aag aag cta aag aaa agg aat aag ccc tgc cca gta tgt aga caa			1440
Ala Lys Lys Leu Lys Lys Arg Asn Lys Pro Cys Pro Val Cys Arg Gln			
465	470	475	480
cca att caa atg att gtg cta act tat ttc ccc tag			1476
Pro Ile Gln Met Ile Val Leu Thr Tyr Phe Pro			
485	490		

<210> 15  
 <211> 491  
 <212> PRT  
 <213> Homo sapiens

<400> 15  
 Met Cys Asn Thr Asn Met Ser Val Pro Thr Asp Gly Ala Val Thr Thr  
 1 5 10 15  
 Ser Gln Ile Pro Ala Ser Glu Gln Glu Thr Leu Val Arg Pro Lys Pro  
 20 25 30  
 Leu Leu Leu Lys Leu Leu Lys Ser Val Gly Ala Gln Lys Asp Thr Tyr  
 35 40 45  
 Thr Met Lys Glu Val Leu Phe Tyr Leu Gly Gln Tyr Ile Met Thr Lys  
 50 55 60  
 Arg Leu Tyr Asp Glu Lys Gln Gln His Ile Val Tyr Cys Ser Asn Asp  
 65 70 75 80  
 Leu Leu Gly Asp Leu Phe Gly Val Pro Ser Phe Ser Val Lys Glu His  
 85 90 95

Arg Lys Ile Tyr Thr Met Ile Tyr Arg Asn Leu Val Val Val Asn Gln  
 100 105 110  
 Gln Glu Ser Ser Asp Ser Gly Thr Ser Val Ser Glu Asn Arg Cys His  
 115 120 125  
 Leu Glu Gly Gly Ser Asp Gln Lys Asp Leu Val Gln Glu Leu Gln Glu  
 130 135 140  
 Glu Lys Pro Ser Ser Ser His Leu Val Ser Arg Pro Ser Thr Ser Ser  
 145 150 155 160  
 Arg Arg Arg Ala Ile Ser Glu Thr Glu Glu Asn Ser Asp Glu Leu Ser  
 165 170 175  
 Gly Glu Arg Gln Arg Lys Arg His Lys Ser Asp Ser Ile Ser Leu Ser  
 180 185 190  
 Phe Asp Glu Ser Leu Ala Leu Cys Val Ile Arg Glu Ile Cys Cys Glu  
 195 200 205  
 Arg Ser Ser Ser Ser Glu Ser Thr Gly Thr Pro Ser Asn Pro Asp Leu  
 210 215 220  
 Asp Ala Gly Val Ser Glu His Ser Gly Asp Trp Leu Asp Gln Asp Ser  
 225 230 235 240  
 Val Ser Asp Gln Phe Ser Val Glu Phe Glu Val Glu Ser Leu Asp Ser  
 245 250 255  
 Glu Asp Tyr Ser Leu Ser Glu Glu Gly Gln Glu Leu Ser Asp Glu Asp  
 260 265 270  
 Asp Glu Val Tyr Gln Val Thr Val Tyr Gln Ala Gly Glu Ser Asp Thr  
 275 280 285  
 Asp Ser Phe Glu Glu Asp Pro Glu Ile Ser Leu Ala Asp Tyr Trp Lys  
 290 295 300  
 Cys Thr Ser Cys Asn Glu Met Asn Pro Pro Leu Pro Ser His Cys Asn  
 305 310 315 320  
 Arg Cys Trp Ala Leu Arg Glu Asn Trp Leu Pro Glu Asp Lys Gly Lys  
 325 330 335  
 Asp Lys Gly Glu Ile Ser Glu Lys Ala Lys Leu Glu Asn Ser Thr Gln  
 340 345 350  
 Ala Glu Glu Gly Phe Asp Val Pro Asp Cys Lys Lys Thr Ile Val Asn  
 355 360 365  
 Asp Ser Arg Glu Ser Cys Val Glu Glu Asn Asp Asp Lys Ile Thr Gln  
 370 375 380  
 Ala Ser Gln Ser Gln Glu Ser Glu Asp Tyr Ser Gln Pro Ser Thr Ser  
 385 390 395 400  
 Ser Ser Ile Ile Tyr Ser Ser Gln Glu Asp Val Lys Glu Phe Glu Arg

405	410	415
Glu Glu Thr Gln Asp Lys Glu Glu Ser Val Glu Ser Ser Leu Pro Leu		
420	425	430
Asn Ala Ile Glu Pro Cys Val Ile Cys Gln Gly Arg Pro Lys Asn Gly		
435	440	445
Cys Ile Val His Gly Lys Thr Gly His Leu Met Ala Cys Phe Thr Cys		
450	455	460
Ala Lys Lys Leu Lys Lys Arg Asn Lys Pro Cys Pro Val Cys Arg Gln		
465	470	475
Pro Ile Gln Met Ile Val Leu Thr Tyr Phe Pro		
485	490	

<210> 16  
 <211> 1317  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> CDS  
 <222> (136)..(1314)  
 <223> Human p53

<400> 16  
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 ggctgggagc gtgctttcca cgacggtgac acgcttcct ggattggcag ccagactgcc 120  
 ttccgggtca ctgcc atg gag gag ccg cag tca gat cct agc gtc gag ccc 171  
                   Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro  
                   1                  5                  10  
 cct ctg agt cag gaa aca ttt tca gac cta tgg aaa cta ctt cct gaa 219  
 Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Leu Leu Pro Glu  
                   15                  20                  25  
 aac aac gtt ctg tcc ccc ttg ccg tcc caa gca atg gat gat ttg atg 267  
 Asn Asn Val Leu Ser Pro Leu Pro Ser Gln Ala Met Asp Asp Leu Met  
                   30                  35                  40  
 ctg tcc ccg gac gat att gaa caa tgg ttc act gaa gac cca ggt cca 315  
 Leu Ser Pro Asp Asp Ile Glu Gln Trp Phe Thr Glu Asp Pro Gly Pro  
                   45                  50                  55                  60  
 gat gaa gct ccc aga atg cca gag gct gct ccc ccc gtg gcc cct gca 363  
 Asp Glu Ala Pro Arg Met Pro Glu Ala Ala Pro Pro Val Ala Pro Ala  
                   65                  70                  75  
 cca gca gct cct aca ccg gcg gcc cct gca cca gcc ccc tcc tgg ccc 411  
 Pro Ala Ala Pro Thr Pro Ala Ala Pro Ala Pro Ala Pro Ser Trp Pro  
                   80                  85                  90

ctg tca tct tct gtc cct tcc cag aaa acc tac cag ggc agc tac ggt	459
Leu Ser Ser Ser Val Pro Ser Gln Lys Thr Tyr Gln Gly Ser Tyr Gly	
95 100 105	
ttc cgt ctg ggc ttc ttg cat tct ggg aca gcc aag tct gtg act tgc	507
Phe Arg Leu Gly Phe Leu His Ser Gly Thr Ala Lys Ser Val Thr Cys	
110 115 120	
acg tac tcc cct gcc ctc aac aag atg ttt tgc caa ctg gcc aag acc	555
Thr Tyr Ser Pro Ala Leu Asn Lys Met Phe Cys Gln Leu Ala Lys Thr	
125 130 135 140	
tgc cct gtg cag ctg tgg gtt gat tcc aca ccc ccg ccc ggc acc cgc	603
Cys Pro Val Gln Leu Trp Val Asp Ser Thr Pro Pro Pro Gly Thr Arg	
145 150 155	
gtc cgc gcc atg gcc atc tac aag cag tca cag cac atg acg gag gtt	651
Val Arg Ala Met Ala Ile Tyr Lys Gln Ser Gln His Met Thr Glu Val	
160 165 170	
gtg agg cgc tgc ccc cac cat gag cgc tgc tca gat agc gat ggt ctg	699
Val Arg Arg Cys Pro His His Glu Arg Cys Ser Asp Ser Asp Gly Leu	
175 180 185	
gcc cct cct cag cat ctt atc cga gtg gaa gga aat ttg cgt gtg gag	747
Ala Pro Pro Gln His Leu Ile Arg Val Glu Gly Asn Leu Arg Val Glu	
190 195 200	
tat ttg gat gac aga aac act ttt cga cat agt gtg gtg gtg ccc tat	795
Tyr Leu Asp Asp Arg Asn Thr Phe Arg His Ser Val Val Val Pro Tyr	
205 210 215 220	
gag ccg cct gag gtt ggc tct gac tgt acc acc atc cac tac aac tac	843
Glu Pro Pro Glu Val Gly Ser Asp Cys Thr Thr Ile His Tyr Asn Tyr	
225 230 235	
atg tgt aac agt tcc tgc atg ggc ggc atg aac cgg agg ccc atc ctc	891
Met Cys Asn Ser Ser Cys Met Gly Gly Met Asn Arg Arg Pro Ile Leu	
240 245 250	
acc atc atc aca ctg gaa gac tcc agt ggt aat cta ctg gga cgg aac	939
Thr Ile Ile Thr Leu Glu Asp Ser Ser Gly Asn Leu Leu Gly Arg Asn	
255 260 265	
agc ttt gag gtg cgt gtt tgt gcc tgt cct ggg aga gac cgg cgc aca	987
Ser Phe Glu Val Arg Val Cys Ala Cys Pro Gly Arg Asp Arg Arg Thr	
270 275 280	
gag gaa gag aat ctc cgc aag aaa ggg gag cct cac cac gag ctg ccc	1035
Glu Glu Glu Asn Leu Arg Lys Lys Gly Glu Pro His His Glu Leu Pro	
285 290 295 300	
cca ggg agc act aag cga gca ctg ccc aac aac acc agc tcc tct ccc	1083
Pro Gly Ser Thr Lys Arg Ala Leu Pro Asn Asn Thr Ser Ser Ser Pro	
305 310 315	
cag cca aag aag aaa cca ctg gat gga gaa tat ttc acc ctt cag atc	1131
Gln Pro Lys Lys Lys Pro Leu Asp Gly Glu Tyr Phe Thr Leu Gln Ile	

320	325	330	
cgt ggg cgt gag cgc ttc gag atg ttc cga gag ctg aat gag gcc ttg			1179
Arg Gly Arg Glu Arg Phe Glu Met Phe Arg Glu Leu Asn Glu Ala Leu			
335	340	345	
gaa ctc aag gat gcc cag gct ggg aag gag cca ggg ggg agc agg gct			1227
Glu Leu Lys Asp Ala Gln Ala Gly Lys Glu Pro Gly Gly Ser Arg Ala			
350	355	360	
cac tcc agc cac ctg aag tcc aaa aag ggt cag tct acc tcc cgc cat			1275
His Ser Ser His Leu Lys Ser Lys Lys Gly Gln Ser Thr Ser Arg His			
365	370	375	380
aaa aaa ctc atg ttc aag aca gaa ggg cct gac tca gac tga			1317
Lys Lys Leu Met Phe Lys Thr Glu Gly Pro Asp Ser Asp			
385	390		

<210> 17  
 <211> 393  
 <212> PRT  
 <213> Homo sapiens

<400> 17  
 Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln  
 1 5 10 15  
 Glu Thr Phe Ser Asp Leu Trp Lys Leu Leu Pro Glu Asn Asn Val Leu  
 20 25 30  
 Ser Pro Leu Pro Ser Gln Ala Met Asp Asp Leu Met Leu Ser Pro Asp  
 35 40 45  
 Asp Ile Glu Gln Trp Phe Thr Glu Asp Pro Gly Pro Asp Glu Ala Pro  
 50 55 60  
 Arg Met Pro Glu Ala Ala Pro Pro Val Ala Pro Ala Pro Ala Ala Pro  
 65 70 75 80  
 Thr Pro Ala Ala Pro Ala Pro Ala Pro Ser Trp Pro Leu Ser Ser Ser  
 85 90 95  
 Val Pro Ser Gln Lys Thr Tyr Gln Gly Ser Tyr Gly Phe Arg Leu Gly  
 100 105 110  
 Phe Leu His Ser Gly Thr Ala Lys Ser Val Thr Cys Thr Tyr Ser Pro  
 115 120 125  
 Ala Leu Asn Lys Met Phe Cys Gln Leu Ala Lys Thr Cys Pro Val Gln  
 130 135 140  
 Leu Trp Val Asp Ser Thr Pro Pro Pro Gly Thr Arg Val Arg Ala Met  
 145 150 155 160  
 Ala Ile Tyr Lys Gln Ser Gln His Met Thr Glu Val Val Arg Arg Cys  
 165 170 175

Pro His His Glu Arg Cys Ser Asp Ser Asp Gly Leu Ala Pro Pro Gln  
 180 185 190

His Leu Ile Arg Val Glu Gly Asn Leu Arg Val Glu Tyr Leu Asp Asp  
 195 200 205

Arg Asn Thr Phe Arg His Ser Val Val Val Pro Tyr Glu Pro Pro Glu  
 210 215 220

Val Gly Ser Asp Cys Thr Thr Ile His Tyr Asn Tyr Met Cys Asn Ser  
 225 230 235 240

Ser Cys Met Gly Gly Met Asn Arg Arg Pro Ile Leu Thr Ile Ile Thr  
 245 250 255

Leu Glu Asp Ser Ser Gly Asn Leu Leu Gly Arg Asn Ser Phe Glu Val  
 260 265 270

Arg Val Cys Ala Cys Pro Gly Arg Asp Arg Arg Thr Glu Glu Glu Asn  
 275 280 285

Leu Arg Lys Lys Gly Glu Pro His His Glu Leu Pro Pro Gly Ser Thr  
 290 295 300

Lys Arg Ala Leu Pro Asn Asn Thr Ser Ser Ser Pro Gln Pro Lys Lys  
 305 310 315 320

Lys Pro Leu Asp Gly Glu Tyr Phe Thr Leu Gln Ile Arg Gly Arg Glu  
 325 330 335

Arg Phe Glu Met Phe Arg Glu Leu Asn Glu Ala Leu Glu Leu Lys Asp  
 340 345 350

Ala Gln Ala Gly Lys Glu Pro Gly Gly Ser Arg Ala His Ser Ser His  
 355 360 365

Leu Lys Ser Lys Lys Gly Gln Ser Thr Ser Arg His Lys Lys Leu Met  
 370 375 380

Phe Lys Thr Glu Gly Pro Asp Ser Asp  
 385 390

<210> 18  
 <211> 579  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> CDS  
 <222> (1) .. (576)  
 <223> Human Bax

<400> 18  
 atg gac ggg tcc ggg gag cag ccc aga ggc ggg ggg ccc acc agc tct 48  
 Met Asp Gly Ser Gly Glu Gln Pro Arg Gly Gly Gly Pro Thr Ser Ser  
 1 5 10 15

18



gag cag atc atg aag aca ggg gcc ctt ttg ctt cag ggt ttc atc cag 96  
 Glu Gln Ile Met Lys Thr Gly Ala Leu Leu Leu Gln Gly Phe Ile Gln  
 20 25 30

gat cga gca ggg cga atg ggg ggg gag gca ccc gag ctg gcc ctg gac 144  
 Asp Arg Ala Gly Arg Met Gly Gly Glu Ala Pro Glu Leu Ala Leu Asp  
 35 40 45

ccg gtg cct cag gat gcg tcc acc aag aag ctg agc gag tgt ctc aag 192  
 Pro Val Pro Gln Asp Ala Ser Thr Lys Lys Leu Ser Glu Cys Leu Lys  
 50 55 60

cgc atc ggg gac gaa ctg gac agt aac atg gag ctg cag agg atg att 240  
 Arg Ile Gly Asp Glu Leu Asp Ser Asn Met Glu Leu Gln Arg Met Ile  
 65 70 75 80

gcc gcc gtg gac aca gac tcc ccc cga gag gtc ttt ttc cga gtg gca 288  
 Ala Ala Val Asp Thr Asp Ser Pro Arg Glu Val Phe Phe Arg Val Ala  
 85 90 95

gct gac atg ttt tct gac ggc aac ttc aac tgg ggc cgg gtt gtc gcc 336  
 Ala Asp Met Phe Ser Asp Gly Asn Phe Asn Trp Gly Arg Val Val Ala  
 100 105 110

ctt ttc tac ttt gcc agc aaa ctg gtg ctc aag gcc ctg tgc acc aag 384  
 Leu Phe Tyr Phe Ala Ser Lys Leu Val Leu Lys Ala Leu Cys Thr Lys  
 115 120 125

gtg ccg gaa ctg atc aga acc atc atg ggc tgg aca ttg gac ttc ctc 432  
 Val Pro Glu Leu Ile Arg Thr Ile Met Gly Trp Thr Leu Asp Phe Leu  
 130 135 140

cgg gag cgg ctg ttg ggc tgg atc caa gac cag ggt ggt tgg gac ggc 480  
 Arg Glu Arg Leu Leu Gly Trp Ile Gln Asp Gln Gly Gly Trp Asp Gly  
 145 150 155 160

ctc ctc tcc tac ttt ggg acg ccc acg tgg cag acc gtg acc atc ttt 528  
 Leu Leu Ser Tyr Phe Gly Thr Pro Thr Trp Gln Thr Val Thr Ile Phe  
 165 170 175

gtg gcg gga gtg ctc acc gcc tcg ctc acc atc tgg aag aag atg ggc 576  
 Val Ala Gly Val Leu Thr Ala Ser Leu Thr Ile Trp Lys Lys Met Gly  
 180 185 190

tga 579

<210> 19  
 <211> 192  
 <212> PRT  
 <213> Homo sapiens

<400> 19  
 Met Asp Gly Ser Gly Glu Gln Pro Arg Gly Gly Gly Pro Thr Ser Ser  
 1 5 10 15  
 Glu Gln Ile Met Lys Thr Gly Ala Leu Leu Leu Gln Gly Phe Ile Gln  
 20 25 30

Asp Arg Ala Gly Arg Met Gly Gly Glu Ala Pro Glu Leu Ala Leu Asp  
           35                          40                          45  
 Pro Val Pro Gln Asp Ala Ser Thr Lys Lys Leu Ser Glu Cys Leu Lys  
           50                          55                          60  
 Arg Ile Gly Asp Glu Leu Asp Ser Asn Met Glu Leu Gln Arg Met Ile  
           65                          70                          75                          80  
 Ala Ala Val Asp Thr Asp Ser Pro Arg Glu Val Phe Phe Arg Val Ala  
                           85                          90                          95  
 Ala Asp Met Phe Ser Asp Gly Asn Phe Asn Trp Gly Arg Val Val Ala  
                           100                          105                          110  
 Leu Phe Tyr Phe Ala Ser Lys Leu Val Leu Lys Ala Leu Cys Thr Lys  
           115                          120                          125  
 Val Pro Glu Leu Ile Arg Thr Ile Met Gly Trp Thr Leu Asp Phe Leu  
           130                          135                          140  
 Arg Glu Arg Leu Leu Gly Trp Ile Gln Asp Gln Gly Gly Trp Asp Gly  
           145                          150                          155                          160  
 Leu Leu Ser Tyr Phe Gly Thr Pro Thr Trp Gln Thr Val Thr Ile Phe  
                           165                          170                          175  
 Val Ala Gly Val Leu Thr Ala Ser Leu Thr Ile Trp Lys Lys Met Gly  
           180                          185                          190

<210> 20  
 <211> 507  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> CDS  
 <222> (1)..(504)  
 <223> Human Bad

<400> 20  
 atg ttc cag atc cca gag ttt gag ccg agt gag cag gaa gac tcc agc 48  
 Met Phe Gln Ile Pro Glu Phe Glu Pro Ser Glu Gln Glu Asp Ser Ser  
       1                          5                          10                          15  
 tct gca gag agg ggc ctg ggc ccc agc ccc gca ggg gac ggg ccc tca 96  
 Ser Ala Glu Arg Gly Leu Gly Pro Ser Pro Ala Gly Asp Gly Pro Ser  
           20                          25                          30  
 ggc tcc ggc aag cat cat cgc cag gcc cca ggc ctc ctg tgg gac gcc 144  
 Gly Ser Gly Lys His His Arg Gln Ala Pro Gly Leu Leu Trp Asp Ala  
           35                          40                          45  
 agt cac cag cag gag cag cca acc agc agc agc cat cat gga ggc gct 192  
 Ser His Gln Gln Glu Gln Pro Thr Ser Ser Ser His His Gly Gly Ala  
           50                          55                          60

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ggg gct gtg gag atc cgg agt cgc cac agc tcc tac ccc gcg ggg acg      240
Gly Ala Val Glu Ile Arg Ser Arg His Ser Ser Tyr Pro Ala Gly Thr
 65                      70                      75                      80

gag gac gac gaa ggg atg ggg gag gag ccc agc ccc ttt cgg ggc cgc      288
Glu Asp Asp Glu Gly Met Gly Glu Glu Pro Ser Pro Phe Arg Gly Arg
                      85                      90                      95

tcg cgc tcg gcg ccc ccc aac ctc tgg gca gca cag cgc tat ggc cgc      336
Ser Arg Ser Ala Pro Pro Asn Leu Trp Ala Ala Gln Arg Tyr Gly Arg
                      100                      105                      110

gag ctc cgg agg atg agt gac gag ttt gtg gac tcc ttt aag aag gga      384
Glu Leu Arg Arg Met Ser Asp Glu Phe Val Asp Ser Phe Lys Lys Gly
                      115                      120                      125

ctt cct cgc ccg aag agc gcg ggc aca gca acg cag atg cgg caa agc      432
Leu Pro Arg Pro Lys Ser Ala Gly Thr Ala Thr Gln Met Arg Gln Ser
                      130                      135                      140

tcc agc tgg acg cga gtc ttc cag tcc tgg tgg gat cgg aac ttg ggc      480
Ser Ser Trp Thr Arg Val Phe Gln Ser Trp Trp Asp Arg Asn Leu Gly
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agg gga agc tcc gcc ccc tcc cag tga      507
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 <212> PRT  
 <213> Homo sapiens

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Gly Ser Gly Lys His His Arg Gln Ala Pro Gly Leu Leu Trp Asp Ala
 35                      40                      45

Ser His Gln Gln Glu Gln Pro Thr Ser Ser Ser His His Gly Gly Ala
 50                      55                      60

Gly Ala Val Glu Ile Arg Ser Arg His Ser Ser Tyr Pro Ala Gly Thr
 65                      70                      75                      80

Glu Asp Asp Glu Gly Met Gly Glu Glu Pro Ser Pro Phe Arg Gly Arg
                      85                      90                      95

Ser Arg Ser Ala Pro Pro Asn Leu Trp Ala Ala Gln Arg Tyr Gly Arg
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Glu Leu Arg Arg Met Ser Asp Glu Phe Val Asp Ser Phe Lys Lys Gly
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<223> Human BCL-2
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 Glu Phe Gly Gly Val Met Cys Val Glu Ser Val Asn Arg Glu Met Ser  
 155 160 165

ccc ctg gtg gac aac atc gcc ctg tgg atg act gag tac ctg aac cgg 582  
 Pro Leu Val Asp Asn Ile Ala Leu Trp Met Thr Glu Tyr Leu Asn Arg  
 170 175 180

cac ctg cac acc tgg atc cag gat aac gga ggc tgg gat gcc ttt gtg 630  
 His Leu His Thr Trp Ile Gln Asp Asn Gly Gly Trp Asp Ala Phe Val  
 185 190 195

gaa ctg tac ggc ccc agc atg cgg cct ctg ttt gat ttc tcc tgg ctg 678  
 Glu Leu Tyr Gly Pro Ser Met Arg Pro Leu Phe Asp Phe Ser Trp Leu  
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tct ctg aag act ctg ctc agt ttg gcc ctg gtg gga gct tgc atc acc 726  
 Ser Leu Lys Thr Leu Leu Ser Leu Ala Leu Val Gly Ala Cys Ile Thr  
 220 225 230

ctg ggt gcc tat ctg ggc cac aag tgaagtcaac atgcctgccc caaacaata 780  
 Leu Gly Ala Tyr Leu Gly His Lys  
 235

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cacacacaca cacaacaatt aacagtcttc aggcataaacg tcgaatcagc tatttactgc 960

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&lt;210&gt; 23

&lt;211&gt; 239

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 23

Met Ala His Ala Gly Arg Thr Gly Tyr Asp Asn Arg Glu Ile Val Met  
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Lys Tyr Ile His Tyr Lys Leu Ser Gln Arg Gly Tyr Glu Trp Asp Ala  
 20 25 30

Gly Asp Val Gly Ala Ala Pro Pro Gly Ala Ala Pro Ala Pro Gly Ile  
 35 40 45

Phe Ser Ser Gln Pro Gly His Thr Pro His Thr Ala Ala Ser Arg Asp  
 50 55 60

Pro Val Ala Arg Thr Ser Pro Leu Gln Thr Pro Ala Ala Pro Gly Ala  
 65 70 75 80

Ala Ala Gly Pro Ala Leu Ser Pro Val Pro Pro Val Val His Leu Thr  
 85 90 95

Leu Arg Gln Ala Gly Asp Asp Phe Ser Arg Arg Tyr Arg Arg Asp Phe  
 100 105 110

26



Ala Glu Met Ser Arg Gln Leu His Leu Thr Pro Phe Thr Ala Arg Gly  
 115 120 125

Arg Phe Ala Thr Val Val Glu Glu Leu Phe Arg Asp Gly Val Asn Trp  
 130 135 140

Gly Arg Ile Val Ala Phe Phe Glu Phe Gly Gly Val Met Cys Val Glu  
 145 150 155 160

Ser Val Asn Arg Glu Met Ser Pro Leu Val Asp Asn Ile Ala Leu Trp  
 165 170 175

Met Thr Glu Tyr Leu Asn Arg His Leu His Thr Trp Ile Gln Asp Asn  
 180 185 190

Gly Gly Trp Asp Ala Phe Val Glu Leu Tyr Gly Pro Ser Met Arg Pro  
 195 200 205

Leu Phe Asp Phe Ser Trp Leu Ser Leu Lys Thr Leu Leu Ser Leu Ala  
 210 215 220

Leu Val Gly Ala Cys Ile Thr Leu Gly Ala Tyr Leu Gly His Lys  
 225 230 235

&lt;210&gt; 24

&lt;211&gt; 1561

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (70)..(1413)

&lt;223&gt; Description of Artificial Sequence:

Beta-TRCP.N/SKP2.C hybrid cDNA and protein

&lt;400&gt; 24

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tcggcgatt atg gac ccg gcc gag gcg gtg ctg caa gag aag gca ctc aag 111

Met Asp Pro Ala Glu Ala Val Leu Gln Lys Ala Leu Lys

1

5

10

ttt atg aca tac aac agc tgt gcc aga ctc tgc tta aac caa gaa aca 159

Phe Met Thr Tyr Asn Ser Cys Ala Arg Leu Cys Leu Asn Gln Glu Thr

15

20

25

30

gta tgt tta gca agc act gct atg aag act gag aat tgt gtg gcc aaa 207

Val Cys Leu Ala Ser Thr Ala Met Lys Thr Glu Asn Cys Val Ala Lys

35

40

45

aca aaa ctt gcc aat ggc act tcc agt atg att gtg ccc aag caa cgg 255

Thr Lys Leu Ala Asn Gly Thr Ser Ser Met Ile Val Pro Lys Gln Arg

50

55

60

aaa ctc tca gca agc tat gaa aag gaa aag gaa ctg tgt gtc aaa tac 303

Lys Leu Ser Ala Ser Tyr Glu Lys Glu Lys Glu Leu Cys Val Lys Tyr

27

65	70	75	
ttt gag cag tgg tca gag tca gat caa gtg gaa ttt gtg gaa cat ctt			351
Phe Glu Gln Trp Ser Glu Ser Asp Gln Val Glu Phe Val Glu His Leu			
80	85	90	
ata tcc caa atg tgt cat tac caa cat ggg cac ata aac tcg tat ctt			399
Ile Ser Gln Met Cys His Tyr Gln His Gly His Ile Asn Ser Tyr Leu			
95	100	105	110
aaa cct atg ttg cag aga gat ttc ata act gct ctg cca gct cgg gga			447
Lys Pro Met Leu Gln Arg Asp Phe Ile Thr Ala Leu Pro Ala Arg Gly			
115	120	125	
ttg gat cat atc gct gag aac att ctg tca tac ctg gat gcc aaa tca			495
Leu Asp His Ile Ala Glu Asn Ile Leu Ser Tyr Leu Asp Ala Lys Ser			
130	135	140	
cta tgt gct gct gaa ctt gtg tgc aag gaa tgg tac cga gtg acc tct			543
Leu Cys Ala Ala Glu Leu Val Cys Lys Glu Trp Tyr Arg Val Thr Ser			
145	150	155	
gat ggc atg ctg tgg aag aag ctt atc gag aga atg gtc agg aca gat			591
Asp Gly Met Leu Trp Lys Lys Leu Ile Glu Arg Met Val Arg Thr Asp			
160	165	170	
tct ctg tgg aga gcc atg gtg tct caa ggg gtg att gcc ttc cgc tgc			639
Ser Leu Trp Arg Ala Met Val Ser Gln Gly Val Ile Ala Phe Arg Cys			
175	180	185	190
cca cga tca ttt atg gac caa cca ttg gct gaa cat ttc agc cct ttt			687
Pro Arg Ser Phe Met Asp Gln Pro Leu Ala Glu His Phe Ser Pro Phe			
195	200	205	
cgt gta cag gac atg gac cta tcg aac tca gtt ata gaa gtg tcc acc			735
Arg Val Gln Asp Met Asp Leu Ser Asn Ser Val Ile Glu Val Ser Thr			
210	215	220	
ctc cac ggc ata ctg tct cag tgt tcc aag ttg cag aat cta agc ctg			783
Leu His Gly Ile Leu Ser Gln Cys Ser Lys Leu Gln Asn Leu Ser Leu			
225	230	235	
gaa ctg cgg ctt tcg gat ccc att gtc aat act ctc gca aaa aac tca			831
Glu Leu Arg Leu Ser Asp Pro Ile Val Asn Thr Leu Ala Lys Asn Ser			
240	245	250	
aat tta gtg cga ctt aac ctt cct ggg tgt cct gga ttc cct aaa ttt			879
Asn Leu Val Arg Leu Asn Leu Pro Gly Cys Pro Gly Phe Pro Lys Phe			
255	260	265	270
ccc ctg cag act ttc cta agc agc tgt ccc aga ctg gat gag ctg aac			927
Pro Leu Gln Thr Phe Leu Ser Ser Cys Pro Arg Leu Asp Glu Leu Asn			
275	280	285	
ctc tcc tgg tgt ttt aat ttc act gaa aag cat gta cag gtg gct gtt			975
Leu Ser Trp Cys Phe Asn Phe Thr Glu Lys His Val Gln Val Ala Val			
290	295	300	

28

gcg cat gtc tca gag acc atg acc cag ctg aat cta agc ggc tac aga 1023  
 Ala His Val Ser Glu Thr Met Thr Gln Leu Asn Leu Ser Gly Tyr Arg  
 305 310 315

aag aat ctc cag aaa tca gat ctc tct act tta gtt aga aga tgc ccc 1071  
 Lys Asn Leu Gln Lys Ser Asp Leu Ser Thr Leu Val Arg Arg Cys Pro  
 320 325 330

aat ctt gtc cat cta gac tta agt aat agt gtc atg cta aag aat gac 1119  
 Asn Leu Val His Leu Asp Leu Ser Asn Ser Val Met Leu Lys Asn Asp  
 335 340 345 350

tgc ttt cag gaa ttt tcc cag ctc aac tac ctc caa cac cta tca ctc 1167  
 Cys Phe Gln Glu Phe Ser Gln Leu Asn Tyr Leu Gln His Leu Ser Leu  
 355 360 365

agt cgg tgc tat gat ata ata cct gaa act tta ctt gaa ctt gga gaa 1215  
 Ser Arg Cys Tyr Asp Ile Ile Pro Glu Thr Leu Leu Glu Leu Gly Glu  
 370 375 380

att ccc aca cta aaa aca cta caa gtt ttt gga atc gtg cca gat ggt 1263  
 Ile Pro Thr Leu Lys Thr Leu Gln Val Phe Gly Ile Val Pro Asp Gly  
 385 390 395

acc ctt caa ctg tta aag gaa gcc ctt cct cat cta cag att aat tgc 1311  
 Thr Leu Gln Leu Leu Lys Glu Ala Leu Pro His Leu Gln Ile Asn Cys  
 400 405 410

tcc cat ttc acc acc att gcc agg cca act att ggc aac aaa aag aac 1359  
 Ser His Phe Thr Thr Ile Ala Arg Pro Thr Ile Gly Asn Lys Lys Asn  
 415 420 425 430

cag gag ata tgg ggc atc aaa tgc cga ctg aca ctg caa aag ccc agt 1407  
 Gln Glu Ile Trp Gly Ile Lys Cys Arg Leu Thr Leu Gln Lys Pro Ser  
 435 440 445

tgt cta tgaagtattt attgcaggat ggtgtctctt ctttagaaca gggaaaatag 1463  
 Cys Leu

gcaggaagcc caattgctgg agtacttagc tagttttatt cttggttttc ccttttgcct 1523

gtcattctgc aagtatacta gggagcccat tttgagag 1561

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<211> 448

<212> PRT

<213> Artificial Sequence

<400> 25

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Thr Tyr Asn Ser Cys Ala Arg Leu Cys Leu Asn Gln Glu Thr Val Cys  
 20 25 30

Leu Ala Ser Thr Ala Met Lys Thr Glu Asn Cys Val Ala Lys Thr Lys  
 35 40 45

29

Leu Ala Asn Gly Thr Ser Ser Met Ile Val Pro Lys Gln Arg Lys Leu  
 50 55 60  
 Ser Ala Ser Tyr Glu Lys Glu Lys Glu Leu Cys Val Lys Tyr Phe Glu  
 65 70 75 80  
 Gln Trp Ser Glu Ser Asp Gln Val Glu Phe Val Glu His Leu Ile Ser  
 85 90 95  
 Gln Met Cys His Tyr Gln His Gly His Ile Asn Ser Tyr Leu Lys Pro  
 100 105 110  
 Met Leu Gln Arg Asp Phe Ile Thr Ala Leu Pro Ala Arg Gly Leu Asp  
 115 120 125  
 His Ile Ala Glu Asn Ile Leu Ser Tyr Leu Asp Ala Lys Ser Leu Cys  
 130 135 140  
 Ala Ala Glu Leu Val Cys Lys Glu Trp Tyr Arg Val Thr Ser Asp Gly  
 145 150 155 160  
 Met Leu Trp Lys Lys Leu Ile Glu Arg Met Val Arg Thr Asp Ser Leu  
 165 170 175  
 Trp Arg Ala Met Val Ser Gln Gly Val Ile Ala Phe Arg Cys Pro Arg  
 180 185 190  
 Ser Phe Met Asp Gln Pro Leu Ala Glu His Phe Ser Pro Phe Arg Val  
 195 200 205  
 Gln Asp Met Asp Leu Ser Asn Ser Val Ile Glu Val Ser Thr Leu His  
 210 215 220  
 Gly Ile Leu Ser Gln Cys Ser Lys Leu Gln Asn Leu Ser Leu Glu Leu  
 225 230 235 240  
 Arg Leu Ser Asp Pro Ile Val Asn Thr Leu Ala Lys Asn Ser Asn Leu  
 245 250 255  
 Val Arg Leu Asn Leu Pro Gly Cys Pro Gly Phe Pro Lys Phe Pro Leu  
 260 265 270  
 Gln Thr Phe Leu Ser Ser Cys Pro Arg Leu Asp Glu Leu Asn Leu Ser  
 275 280 285  
 Trp Cys Phe Asn Phe Thr Glu Lys His Val Gln Val Ala Val Ala His  
 290 295 300  
 Val Ser Glu Thr Met Thr Gln Leu Asn Leu Ser Gly Tyr Arg Lys Asn  
 305 310 315 320  
 Leu Gln Lys Ser Asp Leu Ser Thr Leu Val Arg Arg Cys Pro Asn Leu  
 325 330 335  
 Val His Leu Asp Leu Ser Asn Ser Val Met Leu Lys Asn Asp Cys Phe  
 340 345 350  
 Gln Glu Phe Ser Gln Leu Asn Tyr Leu Gln His Leu Ser Leu Ser Arg

355                      360                      365  
 Cys Tyr Asp Ile Ile Pro Glu Thr Leu Leu Glu Leu Gly Glu Ile Pro  
     370                      375                      380  
 Thr Leu Lys Thr Leu Gln Val Phe Gly Ile Val Pro Asp Gly Thr Leu  
     385                      390                      395                      400  
 Gln Leu Leu Lys Glu Ala Leu Pro His Leu Gln Ile Asn Cys Ser His  
                     405                      410                      415  
 Phe Thr Thr Ile Ala Arg Pro Thr Ile Gly Asn Lys Lys Asn Gln Glu  
                     420                      425                      430  
 Ile Trp Gly Ile Lys Cys Arg Leu Thr Leu Gln Lys Pro Ser Cys Leu  
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<210> 26  
 <211> 1400  
 <212> DNA  
 <213> Homo sapiens

<400> 26  
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<210> 27  
 <211> 466  
 <212> PRT  
 <213> Homo sapiens

<400> 27  
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	25	Asp	Ile
		30	Val
			Thr
			Leu
Cys	Arg	Cys	Ala
35	Gln	Ile	Ser
	Lys	Ala	Trp
	40	Asn	Ile
		45	Leu
			Ala
			Leu
			Asp
Gly	Ser	Asn	Trp
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	55	Asp	Leu
		60	Phe
			Asn
			Phe
			Gln
			Ile
			Asp
			Val
Glu	Gly	Arg	Val
65	Val	Val	Glu
	70	Asn	Ile
		75	Ser
			Lys
			Arg
			Cys
			Gly
			Gly
			Phe
			Leu
			80
Arg	Lys	Leu	Ser
	85	Leu	Arg
		Gly	Cys
		90	Ile
			Gly
			Val
			Gly
			Asp
			Ser
			Ser
			Leu
			95
Lys	Thr	Phe	Ala
	100	Gln	Asn
		Cys	Arg
		105	Asn
			Ile
			Glu
			His
			Leu
			Asn
			Leu
			Asn
			110
Gly	Cys	Thr	Lys
	115	Ile	Thr
		Asp	Ser
		120	Thr
			Cys
			Tyr
			Ser
			Leu
			Ser
			Arg
			Phe
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Cys	Ser	Lys	Leu
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			Leu
			Thr
			Ser
			Cys
			Val
			Ser
			Ile
			Thr
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		150	Ile
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			Glu
			Gly
			Cys
			Arg
			Asn
			Leu
			Glu
			Tyr
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	165	Trp	Cys
		Asp	Gln
			Ile
			Thr
			Lys
			Asp
			Gly
			Ile
			Glu
			Ala
			175
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		Gly	Leu
		185	Lys
			Ala
			Leu
			Leu
			Leu
			Arg
			Gly
			Cys
			190
Thr	Gln	Leu	Glu
	195	Asp	Glu
		Ala	Leu
		200	Lys
			His
			Ile
			Gln
			Asn
			Tyr
			Cys
			His
			205
Glu	Leu	Val	Ser
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		215	Leu
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			Ser
			Cys
			Ser
			Arg
			Ile
			Thr
			Asp
			Glu
			220
Gly	Val	Val	Gln
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		230	Arg
			Gly
			Cys
			His
			Arg
			Leu
			Gln
			Ala
			Leu
			Cys
			240
Leu	Ser	Gly	Cys
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		Leu	Thr
		250	Asp
			Ala
			Ser
			Leu
			Thr
			Ala
			Leu
			Gly
			255
Leu	Asn	Cys	Pro
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		Gln	Ile
		265	Leu
			Glu
			Ala
			Ala
			Arg
			Cys
			Ser
			His
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Leu	Thr	Asp	Ala
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		Thr	Leu
		280	Leu
			Ala
			Arg
			Asn
			Cys
			His
			Glu
			Leu
			285
Glu	Lys	Met	Asp
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		295	Glu
			Cys
			Ile
			Leu
			Ile
			Thr
			Asp
			Ser
			Thr
			Leu
			300
Ile	Gln	Leu	Ser
	305	Ile	His
		310	Cys
			Pro
			Lys
			Leu
			Gln
			Ala
			Leu
			Ser
			Leu
			Ser
			320

His Cys Glu Leu Ile Thr Asp Asp Gly Ile Leu His Leu Ser Asn Ser  
325 330 335

Thr Cys Gly His Glu Arg Leu Arg Val Leu Glu Leu Asp Asn Cys Leu  
340 345 350

Leu Ile Thr Asp Val Ala Leu Glu His Leu Glu Asn Cys Arg Gly Leu  
355 360 365

Glu Arg Leu Glu Leu Tyr Asp Cys Gln Gln Val Thr Arg Ala Gly Ile  
370 375 380

Lys Arg Met Arg Ala Gln Leu Pro His Val Lys Val His Ala Tyr Phe  
385 390 395 400

Ala Pro Val Thr Pro Pro Thr Ala Val Ala Gly Ser Gly Gln Arg Leu  
405 410 415

Cys Arg Cys Cys Val Ile Leu Glx Gln Gln Leu Pro Gly Pro Lys Gly  
420 425 430

Glx Glx Gly Ile Leu Ser Ser Arg Arg Pro Glu Ser Ser Glx Pro Thr  
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Pro Pro Ser Pro Asn Leu Leu Ile Leu His Trp Glu Arg His Leu Gln  
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Val Lys  
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<210> 28

<211> 2797

<212> DNA

<213> Homo sapiens

<400> 28

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gaaaatgaat acattattgg tttgcttcaa caacgcagcc agaccattta taatgtacat 240
tctgacaata aactctccga gatgcttagc ctctttgaaa agggactgaa gaatgttaag 300
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&lt;210&gt; 29

&lt;211&gt; 691

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 29

```

Met Ala Pro Phe Pro Glu Glu Val Asp Val Phe Thr Ala Pro His Trp
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Arg Met Lys Gln Leu Val Gly Leu Tyr Cys Asp Lys Leu Ser Lys Thr
      20              25              30

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Asn Phe Ser Asn Asn Asn Asp Phe Arg Ala Leu Leu Gln Ser Leu Tyr
      35              40              45

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Ala Thr Phe Lys Glu Phe Lys Met His Glu Gln Ile Glu Asn Glu Tyr
      50              55              60

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Ile Ile Gly Leu Leu Gln Gln Arg Ser Gln Thr Ile Tyr Asn Val His
      65              70              75              80

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Ser Asp Asn Lys Leu Ser Glu Met Leu Ser Leu Phe Glu Lys Gly Leu
      85              90              95

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Lys Asn Val Lys Asn Glu Tyr Glu Gln Leu Asn Tyr Ala Lys Gln Leu
      100             105             110

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Lys Glu Arg Leu Glu Ala Phe Thr Arg Asp Phe Leu Pro His Met Lys
      115             120             125

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Glu Glu Glu Glu Val Phe Gln Pro Met Leu Met Glu Tyr Phe Thr Tyr  
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Glu Glu Leu Lys Asp Ile Lys Lys Lys Val Ile Ala Gln His Cys Ser  
 145 150 155 160

Gln Lys Asp Thr Ala Glu Leu Leu Arg Gly Leu Ser Leu Trp Asn His  
 165 170 175

Ala Glu Glu Arg Gln Lys Phe Phe Lys Tyr Ser Val Asp Glu Lys Ser  
 180 185 190

Asp Lys Glu Ala Glu Val Ser Glu His Ser Thr Gly Ile Thr His Leu  
 195 200 205

Pro Pro Glu Val Met Leu Ser Ile Phe Ser Tyr Leu Asn Pro Gln Glu  
 210 215 220

Leu Cys Arg Cys Ser Gln Val Ser Met Lys Trp Ser Gln Leu Thr Lys  
 225 230 235 240

Thr Gly Ser Leu Trp Lys His Leu Tyr Pro Val His Trp Ala Arg Gly  
 245 250 255

Asp Trp Tyr Ser Gly Pro Ala Thr Glu Leu Asp Thr Glu Pro Asp Asp  
 260 265 270

Glu Trp Val Lys Asn Arg Lys Asp Glu Ser Arg Ala Phe His Glu Trp  
 275 280 285

Asp Glu Asp Ala Asp Ile Asp Glu Ser Glu Glu Ser Ala Glu Glu Ser  
 290 295 300

Ile Ala Ile Ser Ile Ala Gln Met Glu Lys Arg Leu Leu His Gly Leu  
 305 310 315 320

Ile His Asn Val Leu Pro Tyr Val Gly Thr Ser Val Lys Thr Leu Val  
 325 330 335

Leu Ala Tyr Ser Ser Ala Val Ser Ser Lys Met Val Arg Gln Ile Leu  
 340 345 350

Glu Leu Cys Pro Asn Leu Glu His Leu Asp Leu Thr Gln Thr Asp Ile  
 355 360 365

Ser Asp Ser Ala Phe Asp Ser Trp Ser Trp Leu Gly Cys Cys Gln Ser  
 370 375 380

Leu Arg His Leu Asp Leu Ser Gly Cys Glu Lys Ile Thr Asp Val Ala  
 385 390 395 400

Leu Glu Lys Ile Ser Arg Ala Leu Gly Ile Leu Thr Ser His Gln Ser  
 405 410 415

Gly Phe Leu Lys Thr Ser Thr Ser Lys Ile Thr Ser Thr Ala Trp Lys  
 420 425 430

Asn Lys Asp Ile Thr Met Gln Ser Thr Lys Gln Tyr Ala Cys Leu His

435	440	445
Asp Leu Thr Asn Lys Gly Ile Gly Glu Glu Ile Asp Asn Glu His Pro 450	455	460
Trp Thr Lys Pro Val Ser Ser Glu Asn Phe Thr Ser Pro Tyr Val Trp 465	470	475 480
Met Leu Asp Ala Glu Asp Leu Ala Asp Ile Glu Asp Thr Val Glu Trp 485	490	495
Arg His Arg Asn Val Glu Ser Leu Cys Val Met Glu Thr Ala Ser Asn 500	505	510
Phe Ser Cys Ser Thr Ser Gly Cys Phe Ser Lys Asp Ile Val Gly Leu 515	520	525
Arg Thr Ser Val Cys Trp Gln Gln His Cys Ala Ser Pro Ala Phe Ala 530	535	540
Tyr Cys Gly His Ser Phe Cys Cys Thr Gly Thr Ala Leu Arg Thr Met 545	550	555 560
Ser Ser Leu Pro Glu Ser Ser Ala Met Cys Arg Lys Ala Ala Arg Thr 565	570	575
Arg Leu Pro Arg Gly Lys Asp Leu Ile Tyr Phe Gly Ser Glu Lys Ser 580	585	590
Asp Gln Glu Thr Gly Arg Val Leu Leu Phe Leu Ser Leu Ser Gly Cys 595	600	605
Tyr Gln Ile Thr Asp His Gly Leu Arg Val Leu Thr Leu Gly Gly Gly 610	615	620
Leu Pro Tyr Leu Glu His Leu Asn Leu Ser Gly Cys Leu Thr Ile Thr 625	630	635 640
Gly Ala Gly Leu Gln Asp Leu Val Ser Ala Cys Pro Ser Leu Asn Asp 645	650	655
Glu Tyr Phe Tyr Tyr Cys Asp Asn Ile Asn Gly Pro His Ala Asp Thr 660	665	670
Ala Ser Gly Cys Gln Asn Leu Gln Cys Gly Phe Arg Ala Cys Cys Arg 675	680	685
Ser Gly Glu 690		

&lt;210&gt; 30

&lt;211&gt; 666

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 30

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aaaaaa

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<210> 31  
 <211> 192  
 <212> PRT  
 <213> Homo sapiens

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 20 25 30  
 Gln Gln Gln Gln Gln Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro Leu  
 35 40 45  
 Pro Gln Glu Arg Asn Asn Val Gly Glu Arg Asp Asp Asp Val Pro Ala  
 50 55 60  
 Asp Met Val Ala Glu Glu Ser Gly Pro Gly Ala Gln Asn Ser Pro Tyr  
 65 70 75 80  
 Gln Leu Arg Arg Lys Thr Leu Leu Pro Lys Arg Thr Ala Cys Pro Thr  
 85 90 95  
 Lys Asn Ser Met Glu Gly Ala Ser Thr Ser Thr Thr Glu Asn Phe Gly  
 100 105 110  
 His Arg Ala Lys Arg Ala Arg Val Ser Gly Lys Ser Gln Asp Leu Ser  
 115 120 125  
 Ala Ala Pro Ala Glu Gln Tyr Leu Gln Glu Lys Leu Pro Asp Glu Val  
 130 135 140  
 Val Leu Lys Ile Phe Ser Tyr Leu Leu Glu Gln Asp Leu Cys Arg Ala  
 145 150 155 160  
 Ala Cys Val Cys Lys Arg Phe Ser Glu Leu Ala Asn Asp Pro Ile Leu  
 165 170 175  
 Trp Leu Gly Glu Val Ala His Ala Tyr Asn Pro Ser Thr Leu Gly Gly  
 180 185 190

<210> 32  
 <211> 1256  
 <212> DNA  
 <213> Mus musculus

<400> 32  
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 atgatggcct ttgcacctcc aaaaagcatc gatggcccca aaatgcagac aaagatgagt 180  
 acctggacac ctctcaacca tcagcttctg aatgaccagg tatttgaaga acgaagagct 240  
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 agagaacttc acgtcaccaa gcccaagaca cctccaaagg atgaattcac aactgccgat 660  
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<210> 33  
 <211> 373  
 <212> PRT  
 <213> Mus musculus

<400> 33  
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 Gly Lys Ile Pro Val His Phe Met Met Ala Phe Ala Pro Pro Lys Ser  
 35 40 45  
 Ile Asp Gly Pro Lys Met Gln Thr Lys Met Ser Thr Trp Thr Pro Leu  
 50 55 60  
 Asn His Gln Leu Leu Asn Asp Gln Val Phe Glu Glu Arg Arg Ala Leu  
 65 70 75 80  
 Leu Gly Lys Trp Phe Asp Lys Trp Thr Asp Ser Gln Arg Arg Arg Ile  
 85 90 95  
 Leu Thr Gly Leu Leu Glu Arg Cys Ser Leu Ser Gln Gln Lys Phe Cys  
 100 105 110  
 Cys Arg Lys Leu Gln Glu Lys Ile Pro Ala Glu Ala Leu Asp Phe Thr  
 115 120 125

Thr Lys Leu Pro Arg Val Leu Ser Val Tyr Ile Phe Ser Phe Leu Asp  
 130 135 140  
 Pro Arg Ser Leu Cys Arg Cys Ala Gln Val Ser Trp Tyr Trp Lys Ser  
 145 150 155 160  
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 Trp Tyr Ile Ser Phe Ser Pro Thr Pro Phe Glu Gln Gly Val Trp Lys  
 180 185 190  
 Lys His Tyr Ile Gln Met Val Arg Glu Leu His Val Thr Lys Pro Lys  
 195 200 205  
 Thr Pro Pro Lys Asp Glu Phe Thr Thr Ala Asp Val Gln Pro Ile Pro  
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 Gly Asn Ser Pro Asp Glu Lys Gln Ser Pro Ser Leu Ala Phe Arg Ser  
 225 230 235 240  
 Ser Ser Ser Leu Arg Lys Lys Asn Asn Pro Gly Glu Lys Glu Leu Pro  
 245 250 255  
 Pro Trp Arg Ser Ser Asp Lys His Pro Thr Asp Ile Ile Arg Phe Asn  
 260 265 270  
 Tyr Leu Asp Asn Cys Asp Pro Glu Leu Phe Arg Leu Gly Arg Arg Lys  
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 Arg Ser Glu Val Thr Pro Asp Phe Lys Arg Gln Leu Arg Asp Lys Lys  
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 305 310 315 320  
 Ser Leu Ser Ser Pro Pro Lys Val Pro Val Arg Leu Ala Trp Pro Leu  
 325 330 335  
 His Leu Pro Val Ala Pro Ser Asp Arg Glu Ala Ala Thr Glu Ala Leu  
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<210> 34  
 <211> 1825  
 <212> DNA  
 <213> Homo sapiens

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 cttatggaga gaaaggactt tgagacatgg cttgataaca tttctgttac atttctttct 180

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tctgcctaata tggttgccca taaaaa 1825

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&lt;210&gt; 35

&lt;211&gt; 422

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 35

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Met Glu Arg Lys Asp Phe Glu Thr Trp Leu Asp Asn Ile Ser Val Thr
  1              5              10              15

```

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Phe Leu Ser Leu Met Asp Leu Gln Lys Asn Glu Thr Leu Asp His Leu
      20              25              30

```

```

Ile Ser Leu Ser Gly Ala Val Gln Leu Arg His Leu Ser Asn Asn Leu
  35              40              45

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Glu Thr Leu Leu Lys Arg Asp Phe Leu Lys Leu Leu Pro Leu Glu Leu
  50              55              60

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```

Ser Phe Tyr Leu Leu Lys Trp Leu Asp Pro Gln Thr Leu Leu Thr Cys
  65              70              75              80

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```

Cys Leu Val Ser Lys Gln Arg Asn Lys Val Ile Ser Ala Cys Thr Glu
      85              90              95

```

```

Val Trp Gln Thr Ala Cys Lys Asn Leu Gly Trp Gln Ile Asp Asp Ser
  100              105              110

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```

Val Gln Asp Ser Leu His Trp Lys Lys Val Tyr Leu Lys Ala Ile Leu

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115	120	125
Arg Met Lys Gln Leu Glu Asp His Glu Ala Phe Glu Thr Ser Ser Leu 130 135 140		
Ile Gly His Ser Ala Arg Val Tyr Ala Leu Tyr Tyr Lys Asp Gly Leu 145 150 155 160		
Leu Cys Thr Gly Ser Asp Asp Leu Ser Ala Lys Leu Trp Asp Val Ser 165 170 175		
Thr Gly Gln Cys Val Tyr Gly Ile Gln Thr His Thr Cys Ala Ala Val 180 185 190		
Lys Phe Asp Glu Gln Lys Leu Val Thr Gly Ser Phe Asp Asn Thr Val 195 200 205		
Ala Cys Trp Glu Trp Ser Ser Gly Ala Arg Thr Gln His Phe Arg Gly 210 215 220		
His Thr Gly Ala Val Phe Ser Val Asp Tyr Ser Asp Glu Leu Asp Ile 225 230 235 240		
Leu Val Ser Gly Ser Ala Asp Phe Ala Val Lys Val Trp Ala Leu Ser 245 250 255		
Ala Gly Thr Cys Leu Asn Thr Leu Thr Gly His Thr Glu Trp Val Thr 260 265 270		
Lys Val Val Leu Gln Lys Cys Lys Val Lys Ser Leu Leu His Ser Pro 275 280 285		
Gly Asp Tyr Ile Leu Leu Ser Ala Asp Lys Tyr Glu Ile Lys Ile Trp 290 295 300		
Pro Ile Gly Arg Glu Ile Asn Cys Lys Cys Leu Lys Thr Leu Ser Val 305 310 315 320		
Ser Glu Asp Arg Ser Ile Cys Leu Gln Pro Arg Leu His Phe Asp Gly 325 330 335		
Lys Tyr Ile Val Cys Ser Ser Ala Leu Gly Leu Tyr Gln Trp Asp Phe 340 345 350		
Ala Ser Tyr Asp Ile Leu Arg Val Ile Lys Thr Pro Glu Val Ala Asn 355 360 365		
Leu Ala Leu Leu Gly Phe Gly Asp Val Phe Ala Leu Leu Phe Asp Asn 370 375 380		
His Tyr Leu Tyr Ile Met Asp Leu Arg Thr Glu Ser Leu Ile Ser Arg 385 390 395 400		
Trp Pro Leu Pro Glu Tyr Arg Lys Ser Lys Arg Gly Thr Ser Phe Leu 405 410 415		
Ala Gly Glu Arg Pro Gly 420		

<210> 36  
 <211> 1847  
 <212> DNA  
 <213> Homo sapiens

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 caggcgccga agaagcggcg acggcccag gctgccgaga ggcgggctcg gcggccggag 180  
 aatgaaatga atgatgtgcc tttctttgat atccaactgc cttacgaatt ggcaatcaat 240  
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 ccgcatagca gcatctctga ctattcttgc tggaaagctca tcttccaaga gtgccgagcc 420  
 aaggaacaca tggtacaaac caactggaag aatcgcaaag gtgccgtgag cgagctggag 480  
 catgttcctg acacagtttt gtgtgatgtg cattctcacg atggtgtggg cattgcggga 540  
 tatacatcag gggatgtgag agtgtgggac acccgcacct gggactacgt agcccccttc 600  
 ctggaatcag aggacgagga ggatgagcct ggaatgcagc caaatgtctc ctttgtgagg 660  
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 atgggtcagt gcaacatgga cgggagggtg aggatccacg acctccgcag tggtaacatc 1260  
 gccctgtcgc tctccgcca tcagctcagg gtctctgctg tgcagatgga tgactggaag 1320  
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<210> 37  
 <211> 531  
 <212> PRT  
 <213> Homo sapiens

<400> 37  
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 Leu Ala Gln Ala Gln Ala Pro Lys Lys Arg Arg Arg Pro Glu Ala Ala  
 20 25 30  
 Glu Arg Arg Ala Arg Arg Pro Glu Asn Glu Met Asn Asp Val Pro Phe  
 35 40 45  
 Phe Asp Ile Gln Leu Pro Tyr Glu Leu Ala Ile Asn Ile Phe Gln Tyr  
 50 55 60



Leu Asp Arg Lys Glu Leu Gly Arg Cys Ala Gln Val Ser Lys Thr Trp  
 65 70 75 80  
 Lys Val Ile Ala Glu Asp Glu Val Leu Trp Tyr Arg Leu Cys Gln Gln  
 85 90 95  
 Glu Gly His Leu Pro Asp Ser Ser Ile Ser Asp Tyr Ser Cys Trp Lys  
 100 105 110  
 Leu Ile Phe Gln Glu Cys Arg Ala Lys Glu His Met Leu Gln Thr Asn  
 115 120 125  
 Trp Lys Asn Arg Lys Gly Ala Val Ser Glu Leu Glu His Val Pro Asp  
 130 135 140  
 Thr Val Leu Cys Asp Val His Ser His Asp Gly Val Val Ile Ala Gly  
 145 150 155 160  
 Tyr Thr Ser Gly Asp Val Arg Val Trp Asp Thr Arg Thr Trp Asp Tyr  
 165 170 175  
 Val Ala Pro Phe Leu Glu Ser Glu Asp Glu Glu Asp Glu Pro Gly Met  
 180 185 190  
 Gln Pro Asn Val Ser Phe Val Arg Ile Asn Ser Ser Leu Ala Val Ala  
 195 200 205  
 Ala Tyr Glu Asp Gly Phe Leu Ile Phe Gly Ile Lys Asp Arg Lys Val  
 210 215 220  
 Pro Cys Ser Ser Phe Glu His Asp Ala Arg Ile Gln Ala Leu Ala Leu  
 225 230 235 240  
 Ser Gln Asp Asp Ala Thr Val Ala Thr Ala Ser Ala Phe Asp Val Val  
 245 250 255  
 Met Leu Ser Pro Asn Glu Glu Gly Tyr Trp Gln Ile Ala Ala Glu Phe  
 260 265 270  
 Glu Val Pro Lys Leu Val Gln Tyr Leu Glu Ile Val Pro Glu Thr Arg  
 275 280 285  
 Arg Tyr Pro Val Ala Val Ala Ala Gly Asp Leu Met Tyr Leu Leu  
 290 295 300  
 Lys Ala Glu Asp Ser Ala Arg Thr Leu Leu Tyr Ala His Gly Pro Pro  
 305 310 315 320  
 Val Thr Cys Leu Asp Val Ser Ala Asn Gln Val Ala Phe Gly Val Gln  
 325 330 335  
 Gly Leu Gly Trp Val Tyr Glu Gly Ser Lys Ile Leu Val Tyr Ser Leu  
 340 345 350  
 Glu Ala Gly Arg Arg Leu Leu Lys Leu Gly Asn Val Leu Arg Asp Phe  
 355 360 365  
 Thr Cys Val Asn Leu Ser Asp Ser Pro Pro Asn Leu Met Val Ser Gly

370                      375                      380  
 Asn Met Asp Gly Arg Val Arg Ile His Asp Leu Arg Ser Gly Asn Ile  
 385                      390                      395                      400  
 Ala Leu Ser Leu Ser Ala His Gln Leu Arg Val Ser Ala Val Gln Met  
                     405                      410                      415  
 Asp Asp Trp Lys Ile Val Ser Gly Gly Glu Glu Gly Leu Val Ser Val  
                     420                      425                      430  
 Trp Asp Tyr Arg Met Asn Gln Lys Leu Trp Lys Val Tyr Ser Gly His  
                     435                      440                      445  
 Pro Val Gln His Ile Ser Phe Ser Ser His Ser Leu Ile Thr Ala Asn  
                     450                      455                      460  
 Val Pro Tyr Gln Thr Val Met Arg Asn Ala Asp Leu Asp Ser Phe Thr  
 465                      470                      475                      480  
 Thr His Arg Arg His Arg Gly Leu Ile Arg Ala Tyr Glu Phe Ala Val  
                     485                      490                      495  
 Asp Gln Leu Ala Phe Gln Ser Pro Leu Pro Val Cys Arg Ser Ser Cys  
                     500                      505                      510  
 Asp Ala Met Ala Thr His Tyr Tyr Asp Leu Ala Leu Ala Phe Pro Tyr  
                     515                      520                      525  
 Asn His Val  
                     530

<210> 38  
 <211> 1684  
 <212> DNA  
 <213> Homo sapiens

<400> 38  
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 gtggcgcgta cgacctcacg ggaggaggtg gatgaggcgg ccagcaccct gacgcggctg 180  
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 ggaagtacaa atcattattg gaatgaaact gtaagagatc caattctgtg gagatacttt 300  
 ttgttgaggg atcttccttc ttggtcttct gttgactgga agtctcttcc agatctagaa 360  
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 tatagaatgt gctgtccata cacaagaaga gcttcaaaat ccagccgtcc tatgtatgga 480  
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 ggaccagggt tggaagaatt gaatacctct ttggtgttga gcttgatgtc ttcagaggaa 600  
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 gaaagagata gagcaaggga agagcatata agtgcagtta acaagatgtt cagtcgacac 780  
 aatgaagggt atgatcaaca aggaagccgg tacagtgtga ttccacagat tcaaaaagtg 840  
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 ttactattaa tagtgtgttc tcacactttt gtgtgtatta ggatcacctg ggaagcttgt 1080  
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aaaa

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&lt;210&gt; 39

&lt;211&gt; 305

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 39

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Gly Ser Glu Pro Arg Ser Gly Thr Asn Ser Pro Pro Pro Phe Ser
  1              5              10              15

Asp Trp Gly Arg Leu Glu Ala Ala Ile Leu Ser Gly Trp Lys Thr Phe
          20              25              30

Trp Gln Ser Val Ser Lys Glu Arg Val Ala Arg Thr Thr Ser Arg Glu
          35              40              45

Glu Val Asp Glu Ala Ala Ser Thr Leu Thr Arg Leu Pro Ile Asp Val
          50              55              60

Gln Leu Tyr Ile Leu Ser Phe Leu Ser Pro His Asp Leu Cys Gln Leu
          65              70              75              80

Gly Ser Thr Asn His Tyr Trp Asn Glu Thr Val Arg Asp Pro Ile Leu
          85              90              95

Trp Arg Tyr Phe Leu Leu Arg Asp Leu Pro Ser Trp Ser Ser Val Asp
          100             105             110

Trp Lys Ser Leu Pro Asp Leu Glu Ile Leu Lys Lys Pro Ile Ser Glu
          115             120             125

Val Thr Asp Gly Ala Phe Phe Asp Tyr Met Ala Val Tyr Arg Met Cys
          130             135             140

Cys Pro Tyr Thr Arg Arg Ala Ser Lys Ser Ser Arg Pro Met Tyr Gly
          145             150             155             160

Ala Val Thr Ser Phe Leu His Ser Leu Ile Ile Gln Asn Glu Pro Arg
          165             170             175

Phe Ala Met Phe Gly Pro Gly Leu Glu Glu Leu Asn Thr Ser Leu Val
          180             185             190

Leu Ser Leu Met Ser Ser Glu Glu Leu Cys Pro Thr Ala Gly Leu Pro
          195             200             205

Gln Arg Gln Ile Asp Gly Ile Gly Ser Gly Val Asn Phe Gln Leu Asn

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210 215 220

Asn Gln His Lys Phe Asn Ile Leu Ile Leu Tyr Ser Thr Thr Arg Lys  
 225 230 235 240

Glu Arg Asp Arg Ala Arg Glu Glu His Thr Ser Ala Val Asn Lys Met  
 245 250 255

Phe Ser Arg His Asn Glu Gly Asp Asp Gln Gln Gly Ser Arg Tyr Ser  
 260 265 270

Val Ile Pro Gln Ile Gln Lys Val Cys Glu Val Val Asp Gly Phe Ile  
 275 280 285

Tyr Val Ala Asn Ala Glu Ala His Lys Ser Lys Tyr Ser Tyr Val His  
 290 295 300

Phe  
 305

<210> 40  
 <211> 1025  
 <212> DNA  
 <213> Homo sapiens

<400> 40

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 tacagtgagc aaggctacct caccagagag cagagcagga gaatggctgc gagcaacatt 180  
 tctaacacca atcatcgtaa acaagtccaa ggaggcattg acatatatca tcttttgaag 240  
 gcaaggaaat cgaaagaaca ggaaggattc attaatgttg aaatggtgcc tcctgagcta 300  
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 tggatgaagg cagcctcacc tttaatgcca acccagatga gggagtgaac tactttatgt 540  
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 cactaaattg gaaaaaactg agaattctatc ttgatgaaag gagagatgtc ttggatgacc 660  
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 cacatagatt ctgtgcttgc aaccctgatt taatgcgaga acttggcctt agtcctgatg 840  
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<210> 41  
 <211> 175  
 <212> PRT  
 <213> Homo sapiens

<400> 41

Lys Ser Pro Pro Arg Gly Gln Phe Val Ala Ala Ala Val Glu Ile Ala  
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Gly Arg Ser Gly Leu Gln Met Gly Gln Gly Leu Trp Arg Val Arg  
 20 25 30

46

Asn Gln Gln Leu Gln Gln Glu Gly Tyr Ser Glu Gln Gly Tyr Leu Thr  
           35                                  40                                  45  
 Arg Glu Gln Ser Arg Arg Met Ala Ala Ser Asn Ile Ser Asn Thr Asn  
           50                                  55                                  60  
 His Arg Lys Gln Val Gln Gly Gly Ile Asp Ile Tyr His Leu Leu Lys  
           65                                  70                                  75                                  80  
 Ala Arg Lys Ser Lys Glu Gln Glu Gly Phe Ile Asn Leu Glu Met Leu  
                                   85                                  90                                  95  
 Pro Pro Glu Leu Ser Phe Thr Ile Leu Ser Tyr Leu Asn Ala Thr Asp  
                                   100                                  105                                  110  
 Leu Cys Leu Ala Ser Cys Val Trp Gln Asp Leu Ala Asn Asp Glu Leu  
                                   115                                  120                                  125  
 Leu Trp Gln Gly Leu Cys Lys Ser Thr Trp Gly His Cys Ser Ile Tyr  
                                   130                                  135                                  140  
 Asn Lys Asn Pro Pro Leu Gly Phe Ser Phe Arg Lys Cys Ile Cys Ser  
           145                                  150                                  155                                  160  
 Trp Met Lys Ala Ala Ser Pro Leu Met Pro Thr Gln Met Arg Glu  
                                   165                                  170                                  175

<210> 42  
 <211> 2151  
 <212> DNA  
 <213> Homo sapiens

<400> 42  
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 ggcacttcca gtatgattgt gcccaagcaa cggaaactct cagcaagcta tgaaaaggaa 360  
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 cctatgttgc agagagattt cataactgct ctgccagctc ggggattgga tcatatcgct 540  
 gagaacattc tgtcatacct ggatgccaaa tcactatgtg ctgctgaact tgtgtgcaag 600  
 gaatggtacc gagtgcctc tgatggcatg ctgtggaaga agcttatcga gagaatggctc 660  
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 cagagaattc actgccgaag tgaaacaagc aaaggagttt actgtttaca gtatgatgat 900  
 cagaaaatag taagcggcct tcgagacaac acaatcaaga tctgggataa aaacacattg 960  
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 tacagggaca ggctggtagt gagtggctca tctgacaaca ctatcagatt atgggacata 1440

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cattccggaa gagtttttcg actacagttt gatgaattcc agattgtcag tagttcacat 1680
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actgactgct tcagtgtctg tatcagaaga tgtcttctat caattgtgaa tgattggaac 2040
ttttaaacct cccctcctct cctcctttca cctctgcacc tagttttttc ccattggttc 2100
cagacaaagg tgacttataa atatatttag tgttttgcca gaaaaaaaaa a 2151

```

&lt;210&gt; 43

&lt;211&gt; 519

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 43

```

Met Asp Pro Ala Glu Ala Val Leu Gln Glu Lys Ala Leu Lys Phe Met
  1              5              10              15

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Asn Ser Ser Glu Arg Glu Asp Cys Asn Asn Gly Glu Pro Pro Arg Lys
      20              25              30

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Ile Ile Pro Glu Lys Asn Ser Leu Arg Gln Thr Tyr Asn Ser Cys Ala
      35              40              45

```

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Arg Leu Cys Leu Asn Gln Glu Thr Val Cys Leu Ala Ser Thr Ala Met
      50              55              60

```

```

Lys Thr Glu Asn Cys Val Ala Lys Thr Lys Leu Ala Asn Gly Thr Ser
      65              70              75              80

```

```

Ser Met Ile Val Pro Lys Gln Arg Lys Leu Ser Ala Ser Tyr Glu Lys
      85              90              95

```

```

Glu Lys Glu Leu Cys Val Lys Tyr Phe Glu Gln Trp Ser Glu Ser Asp
      100             105             110

```

```

Gln Val Glu Phe Val Glu His Leu Ile Ser Gln Met Cys His Tyr Gln
      115             120             125

```

```

His Gly His Ile Asn Ser Tyr Leu Lys Pro Met Leu Gln Arg Asp Phe
      130             135             140

```

```

Ile Thr Ala Leu Pro Ala Arg Gly Leu Asp His Ile Ala Glu Asn Ile
      145             150             155             160

```

```

Leu Ser Tyr Leu Asp Ala Lys Ser Leu Cys Ala Ala Glu Leu Val Cys
      165             170             175

```

```

Lys Glu Trp Tyr Arg Val Thr Ser Asp Gly Met Leu Trp Lys Lys Leu
      180             185             190

```

```

Ile Glu Arg Met Val Arg Thr Asp Ser Leu Trp Arg Gly Leu Ala Glu
      195             200             205

```

Arg Arg Gly Trp Gly Gln Tyr Leu Phe Lys Asn Lys Pro Pro Asp Gly  
 210 215 220  
 Asn Ala Pro Pro Asn Ser Phe Tyr Arg Ala Leu Tyr Pro Lys Ile Ile  
 225 230 235 240  
 Gln Asp Ile Glu Thr Ile Glu Ser Asn Trp Arg Cys Gly Arg His Ser  
 245 250 255  
 Leu Gln Arg Ile His Cys Arg Ser Glu Thr Ser Lys Gly Val Tyr Cys  
 260 265 270  
 Leu Gln Tyr Asp Asp Gln Lys Ile Val Ser Gly Leu Arg Asp Asn Thr  
 275 280 285  
 Ile Lys Ile Trp Asp Lys Asn Thr Leu Glu Cys Lys Arg Ile Leu Thr  
 290 295 300  
 Gly His Thr Gly Ser Val Leu Cys Leu Gln Tyr Asp Glu Arg Val Ile  
 305 310 315 320  
 Ile Thr Gly Ser Ser Asp Ser Thr Val Arg Val Trp Asp Val Asn Thr  
 325 330 335  
 Gly Glu Met Leu Asn Thr Leu Ile His His Cys Glu Ala Val Leu His  
 340 345 350  
 Leu Arg Phe Asn Asn Gly Met Met Val Thr Cys Ser Lys Asp Arg Ser  
 355 360 365  
 Ile Ala Val Trp Asp Met Ala Ser Pro Thr Asp Ile Thr Leu Arg Arg  
 370 375 380  
 Val Leu Val Gly His Arg Ala Ala Val Asn Val Val Asp Phe Asp Asp  
 385 390 395 400  
 Lys Tyr Ile Val Ser Ala Ser Gly Asp Arg Thr Ile Lys Val Trp Asn  
 405 410 415  
 Thr Ser Thr Cys Glu Phe Val Arg Thr Leu Asn Gly His Lys Arg Gly  
 420 425 430  
 Ile Ala Cys Leu Gln Tyr Arg Asp Arg Leu Val Val Ser Gly Ser Ser  
 435 440 445  
 Asp Asn Thr Ile Arg Leu Trp Asp Ile Glu Cys Gly Ala Cys Leu Arg  
 450 455 460  
 Val Leu Glu Gly His Glu Glu Leu Val Arg Cys Ile Arg Phe Asp Asn  
 465 470 475 480  
 Lys Arg Ile Val Ser Gly Ala Tyr Asp Gly Lys Ile Lys Val Trp Asp  
 485 490 495  
 Leu Val Ala Ala Leu Asp Pro Arg Ala Pro Ala Gly Thr Leu Cys Leu  
 500 505 510  
 Arg Thr Leu Val Glu His Ser Gly Arg Val Phe Arg Leu Gln Phe Asp

515                      520                      525

Glu Phe Gln Ile Val Ser Ser Ser His Asp Asp Thr Ile Leu Ile Trp  
      530                      535                      540

Asp Phe Leu Asn Asp Pro Ala Ala Gln Ala Glu Pro Pro Arg Ser Pro  
      545                      550                      555                      560

Ser Arg Thr Tyr Thr Tyr Ile Ser Arg  
                                  565

<210> 44  
 <211> 338  
 <212> DNA  
 <213> Mus musculus

<400> 44  
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 ctggatgcct acagtttgct acaggctgcc caagtgaaca agaactggaa tgaacttgca 120  
 agcagtgatg tctgtggag gaagttgtgt cagaagagat ggctctactg ttacatgttc 180  
 accctaccgc tccatggctt agagacatgg aagcagttct tctttaacaa aacatggcaa 240  
 gaacacgcca agacccgggc aaagccagaa gatttcactt acaaggaatt tcctatggag 300  
 tttgaatttc gggcacatcc atggtatata tcaaggca 338

<210> 45  
 <211> 108  
 <212> PRT  
 <213> Mus musculus

<400> 45  
 Met Glu Ile His Leu Pro Ser Val Pro Met Met Glu Ile Leu Ser Tyr  
      1                      5                      10                      15

Leu Asp Ala Tyr Ser Leu Leu Gln Ala Ala Gln Val Asn Lys Asn Trp  
                                  20                      25                      30

Asn Glu Leu Ala Ser Ser Asp Val Leu Trp Arg Lys Leu Cys Gln Lys  
                                  35                      40                      45

Arg Trp Leu Tyr Cys Tyr Met Phe Thr Leu Pro Leu His Gly Leu Glu  
                                  50                      55                      60

Thr Trp Lys Gln Phe Phe Phe Asn Lys Thr Trp Gln Glu His Ala Lys  
                                  65                      70                      75                      80

Thr Arg Ala Lys Pro Glu Asp Phe Thr Tyr Lys Glu Phe Pro Met Glu  
                                  85                      90                      95

Phe Glu Phe Arg Ala His Pro Trp Tyr Ile Ser Arg  
                                  100                      105

<210> 46  
 <211> 849  
 <212> DNA  
 <213> Homo sapiens

50



&lt;400&gt; 46

```

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ggctggggag accgcattcc cttggaaatc ctggtgcaga ttttcgggtt gttggtggcg 120
gcggacggcc ccatgccctt cctgggcagg gctgcgcgcg tgtgccgccg ctggcaggag 180
gccgcttccc aaccgcgcgt ctggcacacc gtgaccctgt cgtccccgct ggtcggccgg 240
cctgccaaagg gcggggtcaa ggcggagaag aagctccttg cttccctgga gtggcttatg 300
cccaatcggg tttcacagct ccagaggctg accctcatcc actggaagtc tcaggtacac 360
cccgtgttga agctggtagg tgagtgtgtt cctcggctca ctttctcaa gctctccggc 420
tgccacgggtg tgactgctga cgctctgggc atgctagcca aagcctgctg ccagctccat 480
agcctggacc tacagcactc catggtggag tccacagctg tggtgagctt cttggaggag 540
gcagggtccc gaatgcgcaa gttgtggctg acctacagct cccagacgac agccatcctg 600
ggcgcatctg tgggcagctg ctgccccag ctccaggctc tggaggtgag caccggcatc 660
aaccgtaata gcattcccc tcaagctgcct gtcgaggctc tgcagaaagg ctgccctcag 720
ctccagggtg tgccggtgtt gaacctgatg tggctgcccc agcctccggg acgaggggtg 780
gctcccgga caggcttccc tagcctagag gagctctgcc tggcgagctc aacctgcaac 840
tttgtgagc                                     849

```

&lt;210&gt; 47

&lt;211&gt; 283

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 47

```

Ala Ala Ala Pro Ala Pro Ala Pro Thr Pro Thr Pro Glu Glu
 1             5             10             15

Gly Pro Asp Ala Gly Trp Gly Asp Arg Ile Pro Leu Glu Ile Leu Val
      20             25             30

Gln Ile Phe Gly Leu Leu Val Ala Ala Asp Gly Pro Met Pro Phe Leu
      35             40             45

Gly Arg Ala Ala Arg Val Cys Arg Arg Trp Gln Glu Ala Ala Ser Gln
      50             55             60

Pro Ala Leu Trp His Thr Val Thr Leu Ser Ser Pro Leu Val Gly Arg
      65             70             75             80

Pro Ala Lys Gly Gly Val Lys Ala Glu Lys Lys Leu Leu Ala Ser Leu
      85             90             95

Glu Trp Leu Met Pro Asn Arg Phe Ser Gln Leu Gln Arg Leu Thr Leu
     100             105             110

Ile His Trp Lys Ser Gln Val His Pro Val Leu Lys Leu Val Gly Glu
     115             120             125

Cys Cys Pro Arg Leu Thr Phe Leu Lys Leu Ser Gly Cys His Gly Val
     130             135             140

Thr Ala Asp Ala Leu Val Met Leu Ala Lys Ala Cys Cys Gln Leu His
     145             150             155             160

Ser Leu Asp Leu Gln His Ser Met Val Glu Ser Thr Ala Val Val Ser
     165             170             175

Phe Leu Glu Glu Ala Gly Ser Arg Met Arg Lys Leu Trp Leu Thr Tyr

```

180 185 190  
 Ser Ser Gln Thr Thr Ala Ile Leu Gly Ala Leu Leu Gly Ser Cys Cys  
 195 200 205  
 Pro Gln Leu Gln Val Leu Glu Val Ser Thr Gly Ile Asn Arg Asn Ser  
 210 215 220  
 Ile Pro Leu Gln Leu Pro Val Glu Ala Leu Gln Lys Gly Cys Pro Gln  
 225 230 235 240  
 Leu Gln Val Leu Arg Leu Leu Asn Leu Met Trp Leu Pro Lys Pro Pro  
 245 250 255  
 Gly Arg Gly Val Ala Pro Gly Pro Gly Phe Pro Ser Leu Glu Glu Leu  
 260 265 270  
 Cys Leu Ala Ser Ser Thr Cys Asn Phe Val Ser  
 275 280

<210> 48  
 <211> 1320  
 <212> DNA  
 <213> Homo sapiens

<400> 48  
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 gaccgtaatt catcagaaga aggaactgca gagaaatcca agaaactgag gactacaaat 120  
 gagcattctc agacttggtga ttggggtaaat ctccttcagg acattattct ccaagtattt 180  
 aaatatttgc ctcttcttga ccggggtcat gcttcacaag tttgccgcaa ctggaaccag 240  
 gtatttcaca tgcctgactt gtggagatgt tttgaatttg aactgaatca gccagctaca 300  
 tcttatttga aagctaccca tccagagctg atcaaacaga ttattaaaag acattcaaac 360  
 catctacaat atgtcagctt caaggtggac agcagcaagg aatcagctga agcagcttgt 420  
 gatatactat cgcaacttgt gaattgctct ttaaaaacac ttggacttat ttcaactgct 480  
 cgaccaagct tttatggattt accaaagtct cactttatct ctgcactgac agttgtgttc 540  
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 ctcaaagtac tagtggccaa caatagtgat acactcaagc tgttgaaaat gagcagctgt 660  
 cctcatgtct ctccagcagg tatcctttgt gtggctgatc agtgtcacgg ctttaagagaa 720  
 cttagccctga actaccactt attgagtgat gagttgttac ttgcattgtc ttctgaaaaa 780  
 catgttcgat tagaacattt gcgcattgat gtagtcagtg agaatcctgg acagacacac 840  
 ttccatacta ttcagaagag tagctgggat gctttcatca gacattcacc caaagtgaac 900  
 ttagtgatgt attttttttt atatgaagaa gaatttgacc ccttcttttcg ctatgaaata 960  
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 gaagagttaa ttcgcattgc agaacgttgc aaaaatttgt cagctatttg actaggggaa 1140  
 tgtgaagtct catgtagtgc ctttgttgag tttgtgaaga tgtgtggtgg ccgcctatct 1200  
 caattatcca ttatggaaga agtactaatt cctgacccaaa agtatagttt ggagcagatt 1260  
 cactgggaag tgtccaagca tcttggttagg gtgtggtttc ccgacatgat gccacttgg 1320

<210> 49  
 <211> 428  
 <212> PRT  
 <213> Homo sapiens

<400> 49  
 Met Lys Arg Gly Gly Arg Asp Ser Asp Arg Asn Ser Ser Glu Glu Gly

52

1	5	10	15
Thr Ala Glu Lys Ser Lys Lys Leu Arg Thr Thr Asn Glu His Ser Gln	20	25	30
Thr Cys Asp Trp Gly Asn Leu Leu Gln Asp Ile Ile Leu Gln Val Phe	35	40	45
Lys Tyr Leu Pro Leu Leu Asp Arg Ala His Ala Ser Gln Val Cys Arg	50	55	60
Asn Trp Asn Gln Val Phe His Met Pro Asp Leu Trp Arg Cys Phe Glu	65	70	75
Phe Glu Leu Asn Gln Pro Ala Thr Ser Tyr Leu Lys Ala Thr His Pro	85	90	95
Glu Leu Ile Lys Gln Ile Ile Lys Arg His Ser Asn His Leu Gln Tyr	100	105	110
Val Ser Phe Lys Val Asp Ser Ser Lys Glu Ser Ala Glu Ala Ala Cys	115	120	125
Asp Ile Leu Ser Gln Leu Val Asn Cys Ser Leu Lys Thr Leu Gly Leu	130	135	140
Ile Ser Thr Ala Arg Pro Ser Phe Met Asp Leu Pro Lys Ser His Phe	145	150	155
Ile Ser Ala Leu Thr Val Val Phe Val Asn Ser Lys Ser Leu Ser Ser	165	170	175
Leu Lys Ile Asp Asp Thr Pro Val Asp Asp Pro Ser Leu Lys Val Leu	180	185	190
Val Ala Asn Asn Ser Asp Thr Leu Lys Leu Leu Lys Met Ser Ser Cys	195	200	205
Pro His Val Ser Pro Ala Gly Ile Leu Cys Val Ala Asp Gln Cys His	210	215	220
Gly Leu Arg Glu Leu Ala Leu Asn Tyr His Leu Leu Ser Asp Glu Leu	225	230	235
Leu Leu Ala Leu Ser Ser Glu Lys His Val Arg Leu Glu His Leu Arg	245	250	255
Ile Asp Val Val Ser Glu Asn Pro Gly Gln Thr His Phe His Thr Ile	260	265	270
Gln Lys Ser Ser Trp Asp Ala Phe Ile Arg His Ser Pro Lys Val Asn	275	280	285
Leu Val Met Tyr Phe Phe Leu Tyr Glu Glu Glu Phe Asp Pro Phe Phe	290	295	300
Arg Tyr Glu Ile Pro Ala Thr His Leu Tyr Phe Gly Arg Ser Val Ser	305	310	315
			320

Lys Asp Val Leu Gly Arg Val Gly Met Thr Cys Pro Arg Leu Val Glu  
 325 330 335

Leu Val Val Cys Ala Asn Gly Leu Arg Pro Leu Asp Glu Glu Leu Ile  
 340 345 350

Arg Ile Ala Glu Arg Cys Lys Asn Leu Ser Ala Ile Gly Leu Gly Glu  
 355 360 365

Cys Glu Val Ser Cys Ser Ala Phe Val Glu Phe Val Lys Met Cys Gly  
 370 375 380

Gly Arg Leu Ser Gln Leu Ser Ile Met Glu Glu Val Leu Ile Pro Asp  
 385 390 395 400

Gln Lys Tyr Ser Leu Glu Gln Ile His Trp Glu Val Ser Lys His Leu  
 405 410 415

Gly Arg Val Trp Phe Pro Asp Met Met Pro Thr Trp  
 420 425

<210> 50

<211> 394

<212> DNA

<213> Homo sapiens

<400> 50

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 ctccccgaca gccttggtcta ccagatcttc ctgagtttgg gccctgcaga tgtgctggct 180  
 gctgggctgg tatgccgcca atggcaggct gtgtcccggg atgagttctt atggaaggag 240  
 caattctacc gctactacca ggtggctcga gatgtgcccc gccaccaga tctggaacaa 300  
 cgacctgacc atctcactgc tgcacagtgc agatatgagg ccatacaact ggagttacac 360  
 ccagttttcc cagttcaacc aggatgactc actg 394

<210> 51

<211> 96

<212> PRT

<213> Homo sapiens

<400> 51

Met Asp Glu Gly Gly Leu Pro Leu Leu Pro Asp Ser Leu Val Tyr Gln  
 1 5 10 15

Ile Phe Leu Ser Leu Gly Pro Ala Asp Val Leu Ala Ala Gly Leu Val  
 20 25 30

Cys Arg Gln Trp Gln Ala Val Ser Arg Asp Glu Phe Leu Trp Lys Glu  
 35 40 45

Gln Phe Tyr Arg Tyr Tyr Gln Val Ala Arg Asp Val Pro Arg His Pro  
 50 55 60

Asp Leu Glu Gln Arg Pro Asp His Leu Thr Ala Ala Gln Cys Arg Tyr  
 65 70 75 80

Glu Ala Ile Gln Leu Glu Leu His Pro Val Phe Pro Val Gln Pro Gly  
 85 90 95

<210> 52  
 <211> 1684  
 <212> DNA  
 <213> Homo sapiens

<400> 52  
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 gtggcgcgta cgacctcacg ggaggagggtg gatgaggcgg ccagcaccct gacgcggctg 180  
 ccgattgatg tacagctata tattttgtcc tttctttcac ctcagtatct gtgtcagttg 240  
 ggaagtacaa atcattattg gaatgaaact gtaagagatc caattctgtg gagatacttt 300  
 ttgttgaggg atcttccttc ttggtcttct gttgactgga agtctcttcc agatctagaa 360  
 atcttaaaaa agcctatatc tgagggtcact gatggtgcat tttttgacta catggcagtc 420  
 tatagaatgt gctgtccata cacaagaaga gcttcaaaat ccagccgtcc tatgtatgga 480  
 gctgtcactt cttttttaca ctccctgatc attcagaatg aaccacgatt tgctatgttt 540  
 ggaccagggt tggaagaatt gaatacctct ttggtgttga gcttgatgtc ttcagaggaa 600  
 ctttgcccaa cagctgggtt gcctcagagg cagattgatg gtattggatc aggagtcaat 660  
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 aggtagaata taaatacata atatattgta tatatattat agttctctaa tacactgggt 1560  
 tgaatttcta ttcaatttat tttccctgt gatctgttag gcatgaaagc agttgcaatt 1620  
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 aaaa 1684

<210> 53  
 <211> 305  
 <212> PRT  
 <213> Homo sapiens

<400> 53  
 Gly Ser Glu Pro Arg Ser Gly Thr Asn Ser Pro Pro Pro Pro Phe Ser  
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Asp Trp Gly Arg Leu Glu Ala Ala Ile Leu Ser Gly Trp Lys Thr Phe  
 20 25 30

Trp Gln Ser Val Ser Lys Glu Arg Val Ala Arg Thr Thr Ser Arg Glu  
 35 40 45

55

Glu Val Asp Glu Ala Ala Ser Thr Leu Thr Arg Leu Pro Ile Asp Val  
 50 55 60  
 Gln Leu Tyr Ile Leu Ser Phe Leu Ser Pro His Asp Leu Cys Gln Leu  
 65 70 75 80  
 Gly Ser Thr Asn His Tyr Trp Asn Glu Thr Val Arg Asp Pro Ile Leu  
 85 90 95  
 Trp Arg Tyr Phe Leu Leu Arg Asp Leu Pro Ser Trp Ser Ser Val Asp  
 100 105 110  
 Trp Lys Ser Leu Pro Asp Leu Glu Ile Leu Lys Lys Pro Ile Ser Glu  
 115 120 125  
 Val Thr Asp Gly Ala Phe Phe Asp Tyr Met Ala Val Tyr Arg Met Cys  
 130 135 140  
 Cys Pro Tyr Thr Arg Arg Ala Ser Lys Ser Ser Arg Pro Met Tyr Gly  
 145 150 155 160  
 Ala Val Thr Ser Phe Leu His Ser Leu Ile Ile Gln Asn Glu Pro Arg  
 165 170 175  
 Phe Ala Met Phe Gly Pro Gly Leu Glu Glu Leu Asn Thr Ser Leu Val  
 180 185 190  
 Leu Ser Leu Met Ser Ser Glu Glu Leu Cys Pro Thr Ala Gly Leu Pro  
 195 200 205  
 Gln Arg Gln Ile Asp Gly Ile Gly Ser Gly Val Asn Phe Gln Leu Asn  
 210 215 220  
 Asn Gln His Lys Phe Asn Ile Leu Ile Leu Tyr Ser Thr Thr Arg Lys  
 225 230 235 240  
 Glu Arg Asp Arg Ala Arg Glu Glu His Thr Ser Ala Val Asn Lys Met  
 245 250 255  
 Phe Ser Arg His Asn Glu Gly Asp Asp Gln Gln Gly Ser Arg Tyr Ser  
 260 265 270  
 Val Ile Pro Gln Ile Gln Lys Val Cys Glu Val Val Asp Gly Phe Ile  
 275 280 285  
 Tyr Val Ala Asn Ala Glu Ala His Lys Ser Lys Tyr Ser Tyr Val His  
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 Phe  
 305

<210> 54  
 <211> 1624  
 <212> DNA  
 <213> Mus musculus

<400> 54

56

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agtagaatca gctttaagac tgggaaaaca acttcttaca gtgtttttgc tcaaacttcc 1560
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aaaa

```

&lt;210&gt; 55

&lt;211&gt; 408

&lt;212&gt; PRT

&lt;213&gt; Mus musculus

&lt;400&gt; 55

```

Thr Ala Pro Leu Thr Leu Glu Ser Leu Pro Thr Asp Pro Leu Leu Leu
  1              5              10              15

```

```

Ile Leu Ser Phe Leu Asp Tyr Arg Asp Leu Ile Asn Cys Cys Tyr Val
      20              25              30

```

```

Ser Arg Arg Leu Ser Gln Leu Ser Ser His Asp Pro Leu Trp Arg Arg
      35              40              45

```

```

His Cys Lys Lys Tyr Trp Leu Ile Ser Glu Glu Glu Lys Thr Gln Lys
      50              55              60

```

```

Asn Gln Cys Trp Lys Ser Leu Phe Ile Asp Thr Tyr Ser Asp Val Gly
      65              70              75              80

```

```

Arg Tyr Ile Asp His Tyr Ala Ala Ile Lys Lys Ala Trp Asp Asp Leu
      85              90              95

```

```

Lys Lys Tyr Leu Glu Pro Arg Cys Pro Arg Met Val Leu Ser Leu Lys
      100              105              110

```

```

Glu Gly Ala Arg Glu Glu Asp Leu Asp Ala Val Glu Ala Gln Ile Gly

```

57

115	120	125
Cys Lys Leu Pro Asp Asp Tyr Arg Cys Ser Tyr Arg Ile His Asn Gly		
130	135	140
Gln Lys Leu Val Val Pro Gly Leu Leu Gly Ser Met Ala Leu Ser Asn		
145	150	155
His Tyr Arg Ser Glu Asp Leu Leu Asp Val Asp Thr Ala Ala Gly Gly		
	165	170
Phe Gln Gln Arg Gln Gly Leu Lys Tyr Cys Leu Pro Leu Thr Phe Cys		
	180	185
Ile His Thr Gly Leu Ser Gln Tyr Ile Ala Val Glu Ala Ala Glu Gly		
	195	200
Arg Asn Lys Asn Glu Val Phe Tyr Gln Cys Pro Asp Gln Met Ala Arg		
	210	215
Asn Pro Ala Ala Ile Asp Met Phe Ile Ile Gly Ala Thr Phe Thr Asp		
	225	230
Trp Phe Thr Ser Tyr Val Lys Asn Val Val Ser Gly Gly Phe Pro Ile		
	245	250
Ile Arg Asp Gln Ile Phe Arg Tyr Val His Asp Pro Glu Cys Val Ala		
	260	265
Thr Thr Gly Asp Ile Thr Val Ser Val Ser Thr Ser Phe Leu Pro Glu		
	275	280
Leu Ser Ser Val His Pro Pro His Tyr Phe Phe Thr Tyr Arg Ile Arg		
	290	295
Ile Glu Met Ser Lys Asp Ala Leu Pro Glu Lys Ala Cys Gln Leu Asp		
	305	310
Ser Arg Tyr Trp Arg Ile Thr Asn Ala Lys Gly Asp Val Glu Glu Val		
	325	330
Gln Gly Pro Gly Val Val Gly Glu Phe Pro Ile Ile Ser Pro Gly Arg		
	340	345
Val Tyr Glu Tyr Thr Ser Cys Thr Thr Phe Ser Thr Thr Ser Gly Tyr		
	355	360
Met Glu Gly Tyr Tyr Thr Phe His Phe Leu Tyr Phe Lys Asp Lys Ile		
	370	375
Phe Asn Val Ala Ile Pro Arg Phe His Met Ala Cys Pro Thr Phe Arg		
	385	390
Val Ser Ile Ala Arg Leu Val Ser		
	405	

&lt;210&gt; 56

58



&lt;211&gt; 4057

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 56

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 <211> 483  
 <212> PRT  
 <213> Homo sapiens

<400> 57  
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 Ser Glu Asp Ser Asp Leu Ser Met Arg Thr Leu Ser Thr Pro Ser Pro  
 35 40 45  
 Ala Leu Ile Cys Pro Pro Asn Leu Pro Gly Phe Gln Asn Gly Arg Gly  
 50 55 60  
 Ser Ser Thr Ser Ser Ser Ser Ile Thr Gly Glu Thr Val Ala Met Val  
 65 70 75 80  
 His Ser Pro Pro Pro Thr Arg Leu Thr His Pro Leu Ile Arg Leu Ala  
 85 90 95  
 Ser Arg Pro Gln Lys Glu Gln Ala Ser Ile Asp Arg Leu Pro Asp His  
 100 105 110  
 Ser Met Val Gln Ile Phe Ser Phe Leu Pro Thr Asn Gln Leu Cys Arg  
 115 120 125  
 Cys Ala Arg Val Cys Arg Arg Trp Tyr Asn Leu Ala Trp Asp Pro Arg  
 130 135 140  
 Leu Trp Arg Thr Ile Arg Leu Thr Gly Glu Thr Ile Asn Val Asp Arg  
 145 150 155 160  
 Ala Leu Lys Val Leu Thr Arg Arg Leu Cys Gln Asp Thr Pro Asn Val  
 165 170 175  
 Cys Leu Met Leu Glu Thr Val Thr Val Ser Gly Cys Arg Arg Leu Thr  
 180 185 190

Asp Arg Gly Leu Tyr Thr Ile Ala Gln Cys Cys Pro Glu Leu Arg Arg  
 195 200 205  
 Leu Glu Val Ser Gly Cys Tyr Asn Ile Ser Asn Glu Ala Val Phe Asp  
 210 215 220  
 Val Val Ser Leu Cys Pro Asn Leu Glu His Leu Asp Val Ser Gly Cys  
 225 230 235 240  
 Ser Lys Val Thr Cys Ile Ser Leu Thr Arg Glu Ala Ser Ile Lys Leu  
 245 250 255  
 Ser Pro Leu His Gly Lys Gln Ile Ser Ile Arg Tyr Leu Asp Met Thr  
 260 265 270  
 Asp Cys Phe Val Leu Glu Asp Glu Gly Leu His Thr Ile Ala Ala His  
 275 280 285  
 Cys Thr Gln Leu Thr His Leu Tyr Leu Arg Arg Cys Val Arg Leu Thr  
 290 295 300  
 Asp Glu Gly Leu Arg Tyr Leu Val Ile Tyr Cys Ala Ser Ile Lys Glu  
 305 310 315 320  
 Leu Ser Val Ser Asp Cys Arg Phe Val Ser Asp Phe Gly Leu Arg Glu  
 325 330 335  
 Ile Ala Lys Leu Glu Ser Arg Leu Arg Tyr Leu Ser Ile Ala His Cys  
 340 345 350  
 Gly Arg Val Thr Asp Val Gly Ile Arg Tyr Val Ala Lys Tyr Cys Ser  
 355 360 365  
 Lys Leu Arg Tyr Leu Asn Ala Arg Gly Cys Glu Gly Ile Thr Asp His  
 370 375 380  
 Gly Val Glu Tyr Leu Ala Lys Asn Cys Thr Lys Leu Lys Ser Leu Asp  
 385 390 395 400  
 Ile Gly Lys Cys Pro Leu Val Ser Asp Thr Gly Leu Glu Cys Leu Ala  
 405 410 415  
 Leu Asn Cys Phe Asn Leu Lys Arg Leu Ser Leu Lys Ser Cys Glu Ser  
 420 425 430  
 Ile Thr Gly Gln Gly Leu Gln Ile Val Ala Ala Asn Cys Phe Asp Leu  
 435 440 445  
 Gln Thr Leu Asn Val Gln Asp Cys Glu Val Ser Val Glu Ala Leu Arg  
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 Ala Phe Phe

<210> 58  
 <211> 516  
 <212> DNA  
 <213> Homo sapiens

<400> 58  
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 atcattcgat aaatgctaatt ttatgactat gttatc 516

<210> 59  
 <211> 160  
 <212> PRT  
 <213> Homo sapiens

<400> 59  
 Met Ala Thr Leu Val Glu Leu Pro Asp Ser Val Leu Leu Glu Ile Phe  
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 20 25 30  
 Arg Trp Lys Arg Leu Val Asp Asp Arg Trp Leu Trp Arg His Val Asp  
 35 40 45  
 Leu Thr Leu Tyr Thr Val Arg Ala Ala Gly Arg Ala Gly Leu Gly Arg  
 50 55 60  
 Gly Arg Gly Ala Arg Thr Pro Lys Thr Thr Ser Pro Thr Leu Gly Leu  
 65 70 75 80  
 Cys Val Gly Ala Arg Ala Gly Asp Leu Glu Pro Gly Asp Pro Gly Pro  
 85 90 95  
 Ile Ser Ala Ser Ser Leu Thr Ser Ser Ser Ser Thr Ser Gly Pro Gln  
 100 105 110  
 Phe Pro His Pro Cys Asn Gly Asp Lys Gly Gln Tyr Leu Pro His Gly  
 115 120 125  
 Ala Ile Met Gly Leu His Glx Asp His Ala Gly Lys Gly Leu Arg Thr  
 130 135 140  
 Val Trp His Ile Phe Ile Ile Arg Glx Met Leu Ile Tyr Asp Tyr Val  
 145 150 155 160

<210> 60

62

<211> 1590  
 <212> DNA  
 <213> Homo sapiens

<400> 60  
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 ggggctgagg cgggagcgag gacacgcccc agagaggaag cagagggagg cggaaagcgtg 180  
 gaggaagggg cgagaggcat catcaaagga gatgagggga gcgtaggggc cgggaaagag 240  
 gcacaaggaa gaaagtatgg gaaggaggaa tggagggtca gggctaggcg gcgggaggggc 300  
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 cacttcacca actgcgacct gctccggcgc cagatagcct gggcctcgct caactccggc 600  
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<210> 61  
 <211> 529  
 <212> PRT  
 <213> Homo sapiens

<400> 61  
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 Asp Gly Glu Gly Gly Ser Gly Pro Gly Ala Glu Ala Gly Ala Arg Thr  
 35 40 45  
 Arg Pro Arg Glu Glu Ala Glu Gly Gly Gly Ser Val Glu Glu Gly Ala  
 50 55 60  
 Arg Gly Ile Ile Lys Gly Asp Glu Gly Ser Val Gly Ala Gly Lys Glu  
 65 70 75 80  
 Ala Gln Gly Arg Lys Tyr Gly Lys Glu Glu Trp Arg Val Arg Ala Arg  
 85 90 95

Arg Arg Glu Gly Ala Arg Pro Gly Arg Val Gln Gly Gln Gly Gly Gln  
 100 105 110  
 Val Trp Ala Tyr Ile Pro Gly Thr Gly Ala Ala Met Ala Ala Ala Ala  
 115 120 125  
 Arg Glu Glu Glu Glu Glu Ala Ala Arg Glu Ser Ala Ala Cys Pro Ala  
 130 135 140  
 Ala Gly Pro Ala Leu Trp Arg Leu Pro Glu Val Leu Leu Leu His Met  
 145 150 155 160  
 Cys Ser Tyr Leu Asp Met Arg Ala Leu Gly Arg Leu Ala Gln Val Tyr  
 165 170 175  
 Arg Trp Leu Trp His Phe Thr Asn Cys Asp Leu Leu Arg Arg Gln Ile  
 180 185 190  
 Ala Trp Ala Ser Leu Asn Ser Gly Phe Thr Arg Leu Gly Thr Asn Leu  
 195 200 205  
 Met Thr Ser Val Pro Val Lys Val Ser Gln Asn Trp Ile Val Gly Cys  
 210 215 220  
 Cys Arg Glu Gly Ile Leu Leu Lys Trp Arg Cys Ser Gln Met Pro Trp  
 225 230 235 240  
 Met Gln Leu Glu Asp Asp Ala Leu Tyr Ile Ser Gln Ala Asn Phe Ile  
 245 250 255  
 Leu Ala Tyr Gln Phe Arg Pro Asp Gly Ala Ser Leu Asn Arg Gln Pro  
 260 265 270  
 Leu Gly Val Ser Ala Gly His Asp Glu Asp Val Cys His Phe Val Leu  
 275 280 285  
 Ala Thr Ser His Ile Val Ser Ala Gly Gly Asp Gly Lys Ile Gly Leu  
 290 295 300  
 Gly Lys Ile His Ser Thr Phe Ala Ala Lys Tyr Trp Ala His Glu Gln  
 305 310 315 320  
 Glu Val Asn Cys Val Asp Cys Lys Gly Gly Ile Ile Ser Phe Gly Ser  
 325 330 335  
 Arg Asp Arg Thr Ala Lys Val Trp Pro Leu Ala Ser Gly Gln Leu Gly  
 340 345 350  
 Gln Cys Leu Tyr Thr Ile Gln Thr Glu Asp Gln Ile Trp Ser Val Ala  
 355 360 365  
 Ile Arg Pro Leu Leu Ser Ser Phe Val Thr Gly Thr Ala Cys Cys Gly  
 370 375 380  
 His Phe Ser Pro Leu Lys Ile Trp Asp Leu Asn Ser Gly Gln Leu Met  
 385 390 395 400  
 Thr His Leu Asp Arg Asp Phe Pro Pro Arg Ala Gly Val Leu Asp Val

405										410					415				
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Val	Arg	Tyr	Trp	Asp	Cys	Arg	Thr	Ser	Val	Arg	Lys	Cys	Val	Met	Glu				
		435					440					445							
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Leu	Tyr	Ala	Ala	Leu	Ser	Tyr	Asn	Leu	His	Val	Leu	Asp	Ile	Gln	Asn				
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Pro

&lt;210&gt; 62

&lt;211&gt; 1680

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 62

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taagtgggtc aagtacacca gccacctcca gacaccagt cgtgctcccg atgctgctat 1500

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65

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<210> 63  
 <211> 395  
 <212> PRT  
 <213> Homo sapiens

<400> 63  
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 20 25 30  
 Lys Ile Asp Arg Thr Ala Arg Asp Gln Cys Gly Ser Gln Pro Trp Asp  
 35 40 45  
 Asn Asn Ala Val Cys Ala Asp Pro Cys Ser Leu Ile Pro Thr Pro Asp  
 50 55 60  
 Lys Glu Asp Asp Asp Arg Val Tyr Pro Asn Ser Thr Cys Lys Pro Arg  
 65 70 75 80  
 Ile Ile Ala Pro Ser Arg Gly Ser Pro Leu Pro Val Leu Ser Trp Ala  
 85 90 95  
 Asn Arg Glu Glu Val Trp Lys Ile Met Leu Asn Lys Glu Lys Thr Tyr  
 100 105 110  
 Leu Arg Asp Gln His Phe Leu Glu Gln His Pro Leu Leu Gln Pro Lys  
 115 120 125  
 Met Arg Ala Ile Leu Leu Asp Trp Leu Met Glu Val Cys Glu Val Tyr  
 130 135 140  
 Lys Leu His Arg Glu Thr Phe Tyr Leu Ala Gln Asp Phe Phe Asp Arg  
 145 150 155 160  
 Tyr Met Ala Thr Gln Glu Asn Val Val Lys Thr Leu Leu Gln Leu Ile  
 165 170 175  
 Gly Ile Ser Ser Leu Phe Ile Ala Ala Lys Leu Glu Glu Ile Tyr Pro  
 180 185 190  
 Pro Lys Leu His Gln Phe Ala Tyr Val Thr Asp Gly Ala Cys Ser Gly  
 195 200 205  
 Asp Glu Ile Leu Thr Met Glu Leu Met Ile Met Lys Ala Leu Lys Trp  
 210 215 220  
 Arg Leu Ser Pro Leu Thr Ile Val Ser Trp Leu Asn Val Tyr Met Gln  
 225 230 235 240  
 Val Ala Tyr Leu Asn Asp Leu His Glu Val Leu Leu Pro Gln Tyr Pro  
 245 250 255

66



Gln Gln Ile Phe Ile Gln Ile Ala Glu Leu Leu Asp Leu Cys Val Leu  
 260 265 270

Asp Val Asp Cys Leu Glu Phe Pro Tyr Gly Ile Leu Ala Ala Ser Ala  
 275 280 285

Leu Tyr His Phe Ser Ser Ser Glu Leu Met Gln Lys Val Ser Gly Tyr  
 290 295 300

Gln Trp Cys Asp Ile Glu Asn Cys Val Lys Trp Met Val Pro Phe Ala  
 305 310 315 320

Met Val Ile Arg Glu Thr Gly Ser Ser Lys Leu Lys His Phe Arg Gly  
 325 330 335

Val Ala Asp Glu Asp Ala His Asn Ile Gln Thr His Arg Asp Ser Leu  
 340 345 350

Asp Leu Leu Asp Lys Ala Arg Ala Lys Lys Ala Met Leu Ser Glu Gln  
 355 360 365

Asn Arg Ala Ser Pro Leu Pro Ser Gly Leu Leu Thr Pro Pro Gln Ser  
 370 375 380

Gly Lys Lys Gln Ser Ser Gly Pro Glu Met Ala  
 385 390 395

<210> 64  
 <211> 597  
 <212> DNA  
 <213> Homo sapiens

<400> 64  
 atgtcaaacg tgcgagtgtc taacggggagc cctagcctgg agcggatgga cgccaggcag 60  
 gcggagcacc ccaagccctc ggccctgcagg aacctcttcg gcccggtgga ccacgaagag 120  
 ttaaccgagg acttgagaa gcaactgcaga gacatggaag aggcgagcca gcgcaagtgg 180  
 aatttcgatt ttcagaatca caaaccctta gagggcaagt acgagtggca agaggtggag 240  
 aagggcagct tgcccagatt ctactacaga ccccgcggc ccccaaaagg tgcctgcaag 300  
 gtgccggcgc aggagagcca ggatgtcagc gggagccgcc cggcggcgcc ttttaattggg 360  
 gctccggcta actctgagga cagcatttg gtggaccaa agactgatcc gtcggacagc 420  
 cagacggggt tagcggagca atgcgcagga ataaggaagc gacctgcaac cgacgattct 480  
 tctactcaaa acaaaagagc caacagaaca gaagaaaatg tttcagacgg ttccccaat 540  
 gccggttctg tggagcagac gcccaagaag cctggcctca gaagacgtca aacgtaa 597

<210> 65  
 <211> 198  
 <212> PRT  
 <213> Homo sapiens

<400> 65  
 Met Ser Asn Val Arg Val Ser Asn Gly Ser Pro Ser Leu Glu Arg Met  
 1 5 10 15

Asp Ala Arg Gln Ala Glu His Pro Lys Pro Ser Ala Cys Arg Asn Leu  
 20 25 30

67

Phe Gly Pro Val Asp His Glu Glu Leu Thr Arg Asp Leu Glu Lys His  
           35                                  40                                  45  
 Cys Arg Asp Met Glu Glu Ala Ser Gln Arg Lys Trp Asn Phe Asp Phe  
           50                                  55                                  60  
 Gln Asn His Lys Pro Leu Glu Gly Lys Tyr Glu Trp Gln Glu Val Glu  
           65                                  70                                  75                                  80  
 Lys Gly Ser Leu Pro Glu Phe Tyr Tyr Arg Pro Pro Arg Pro Pro Lys  
                                   85                                  90                                  95  
 Gly Ala Cys Lys Val Pro Ala Gln Glu Ser Gln Asp Val Ser Gly Ser  
                                   100                                  105                                  110  
 Arg Pro Ala Ala Pro Leu Ile Gly Ala Pro Ala Asn Ser Glu Asp Thr  
                                   115                                  120                                  125  
 His Leu Val Asp Pro Lys Thr Asp Pro Ser Asp Ser Gln Thr Gly Leu  
           130                                  135                                  140  
 Ala Glu Gln Cys Ala Gly Ile Arg Lys Arg Pro Ala Thr Asp Asp Ser  
           145                                  150                                  155                                  160  
 Ser Thr Gln Asn Lys Arg Ala Asn Arg Thr Glu Glu Asn Val Ser Asp  
                                   165                                  170                                  175  
 Gly Ser Pro Asn Ala Gly Ser Val Glu Gln Thr Pro Lys Lys Pro Gly  
                                   180                                  185                                  190  
 Leu Arg Arg Arg Gln Thr  
                                   195

<210> 66  
 <211> 1600  
 <212> DNA  
 <213> Homo sapiens

<400> 66  
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 gtctggctgc tggaggcccg agcagcacgc tcgagccgac gcgcgccaaa gcgggaatct 120  
 ggaaggcgaa gcagctctgc aagtttaatg cacgtattta aaactcccgg gcctgcggac 180  
 gctatgcaca ggaagcacct ccaggagatt ccagacctga gtagcaacgt tgccaccagc 240  
 ttcacgtggg gatgggatc cagcaagact tctgaactgc tgtcaggcat gggggtctcc 300  
 gccctggaga aagaggagcc cgacagttag aacatcccc aggaactgct ctcaaacttg 360  
 ggccaccccg agagccccc acggaacgg ctgaagagca aagggagtga caaagacttt 420  
 gtaattgtcc gcaggcctaa gctaaatcgg gagaactttc caggtgtttc atgggactct 480  
 cttccggatg agctgctctt gggaatcttt tcctgtctgt gcctccctga gctgctaaag 540  
 gtctctgggtg tttgtaagag gtggtatcgc ctacgctctg atgagctctc atggcagacc 600  
 ttagacctta caggtaaaaa tctgcacccg gatgtgactg gtcggttgct gtctcaagg 660  
 gtgattgcct tccgctgccc acgatcattt atggaccaac cattggctga acatttcagc 720  
 ccttttcgtg tacaggacat ggacctatcg aactcagtta tagaagtgtc caccctccac 780  
 ggcatactgt ctacgtgttc caagttgcag aatctaagcc tggaaactgcg gctttcggat 840  
 cccattgtca atactctgcg aaaaaactca aatttagtgc gacttaacct tcctgggtgt 900  
 cctggattcc cttaaatttcc cctgcagact ttcctaagca gctgtcccag actggatgag 960  
 ctgaacctct cctgggtgtt taatttcact gaaaagcatg tacaggtggc tgttgcgcat 1020  
 gtctcagaga ccatgaccca gctgaatcta agcggctaca gaaagaatct ccagaaatca 1080

68

```

gatctctcta ctttagttag aagatgcccc aatcttgtcc atctagactt aagtaatagt 1140
gtcatgctaa agaatgactg ctttcaggaa ttttcccagc tcaactacct ccaacaccta 1200
tcactcagtc ggtgctatga tataataacct gaaactttac ttgaacttgg agaaattccc 1260
acactaaaaa cactacaagt ttttggaatc gtgccagatg gtacccttca actgttaaag 1320
gaagcccttc ctcacttaca gattaattgc tcccatttca ccaccattgc caggccaact 1380
attggcaaca aaaagaacca ggagatatgg ggcattcaat gccgactgac actgcaaaag 1440
cccagttgtc tatgaagtat ttattgcagg atggtgtctc ttcttttagaa cagggaaaat 1500
aggcaggaag cccaattgct ggagtactta gctagtttta ttcttggttt tcccttttgc 1560
ctgtcattct gcaagtatac tagggagccc attttgagag 1600

```

&lt;210&gt; 67

&lt;211&gt; 435

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 67

```

Met His Val Phe Lys Thr Pro Gly Pro Ala Asp Ala Met His Arg Lys
  1              5              10              15

```

```

His Leu Gln Glu Ile Pro Asp Leu Ser Ser Asn Val Ala Thr Ser Phe
      20              25              30

```

```

Thr Trp Gly Trp Asp Ser Ser Lys Thr Ser Glu Leu Leu Ser Gly Met
      35              40              45

```

```

Gly Val Ser Ala Leu Glu Lys Glu Glu Pro Asp Ser Glu Asn Ile Pro
      50              55              60

```

```

Gln Glu Leu Leu Ser Asn Leu Gly His Pro Glu Ser Pro Pro Arg Lys
      65              70              75              80

```

```

Arg Leu Lys Ser Lys Gly Ser Asp Lys Asp Phe Val Ile Val Arg Arg
      85              90              95

```

```

Pro Lys Leu Asn Arg Glu Asn Phe Pro Gly Val Ser Trp Asp Ser Leu
      100             105             110

```

```

Pro Asp Glu Leu Leu Leu Gly Ile Phe Ser Cys Leu Cys Leu Pro Glu
      115             120             125

```

```

Leu Leu Lys Val Ser Gly Val Cys Lys Arg Trp Tyr Arg Leu Ala Ser
      130             135             140

```

```

Asp Glu Ser Leu Trp Gln Thr Leu Asp Leu Thr Gly Lys Asn Leu His
      145             150             155             160

```

```

Pro Asp Val Thr Gly Arg Leu Leu Ser Gln Gly Val Ile Ala Phe Arg
      165             170             175

```

```

Cys Pro Arg Ser Phe Met Asp Gln Pro Leu Ala Glu His Phe Ser Pro
      180             185             190

```

```

Phe Arg Val Gln Asp Met Asp Leu Ser Asn Ser Val Ile Glu Val Ser
      195             200             205

```

```

Thr Leu His Gly Ile Leu Ser Gln Cys Ser Lys Leu Gln Asn Leu Ser
      210             215             220

```

Leu Glu Leu Arg Leu Ser Asp Pro Ile Val Asn Thr Leu Ala Lys Asn  
 225 230 235 240  
 Ser Asn Leu Val Arg Leu Asn Leu Pro Gly Cys Pro Gly Phe Pro Lys  
 245 250 255  
 Phe Pro Leu Gln Thr Phe Leu Ser Ser Cys Pro Arg Leu Asp Glu Leu  
 260 265 270  
 Asn Leu Ser Trp Cys Phe Asn Phe Thr Glu Lys His Val Gln Val Ala  
 275 280 285  
 Val Ala His Val Ser Glu Thr Met Thr Gln Leu Asn Leu Ser Gly Tyr  
 290 295 300  
 Arg Lys Asn Leu Gln Lys Ser Asp Leu Ser Thr Leu Val Arg Arg Cys  
 305 310 315 320  
 Pro Asn Leu Val His Leu Asp Leu Ser Asn Ser Val Met Leu Lys Asn  
 325 330 335  
 Asp Cys Phe Gln Glu Phe Ser Gln Leu Asn Tyr Leu Gln His Leu Ser  
 340 345 350  
 Leu Ser Arg Cys Tyr Asp Ile Ile Pro Glu Thr Leu Leu Glu Leu Gly  
 355 360 365  
 Glu Ile Pro Thr Leu Lys Thr Leu Gln Val Phe Gly Ile Val Pro Asp  
 370 375 380  
 Gly Thr Leu Gln Leu Leu Lys Glu Ala Leu Pro His Leu Gln Ile Asn  
 385 390 395 400  
 Cys Ser His Phe Thr Thr Ile Ala Arg Pro Thr Ile Gly Asn Lys Lys  
 405 410 415  
 Asn Gln Glu Ile Trp Gly Ile Lys Cys Arg Leu Thr Leu Gln Lys Pro  
 420 425 430  
 Ser Cys Leu  
 435

&lt;210&gt; 68

&lt;211&gt; 1455

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 68

cggccgctcg acgctgtagt ggcttcgtct tcggtttttc tcttccttcg ctaacgcctc 60  
 ccggtctctcgc tcagcctccc gccggccgctc tccttaacac cgaacacccat gccttcaatt 120  
 aagttgcaga gttctgatgg agagatattt gaagttgatg tggaaattgc caaacaattct 180  
 gtgactatta agaccatggt ggaagatttg ggaatggatg atgaaggaga tgatgaccca 240  
 gttcctctac caaatgtgaa tgcagcaata ttaaaaaagg tcattcagtg gtgcacccac 300  
 cacaaggatg accctcctcc tcctgaagat gatgagaaca aagaaaagcg aacagatgat 360  
 atccctggtt gggaccaaga attcctgaaa gttgaccaag gaacactttt tgaactcatt 420  
 ctggctgcaa actacttaga catcaaagggt ttgcttgatg ttacatgcaa gactgttgcc 480  
 aatatgatca aggggaaaac tcctgaggag attcgcaaga ctttcaatat caaaaatgac 540

```

tttactgaag aggaggaagc ccagggtacgc aaagagaacc agtgggtgtga agagaagtga 600
aatggtgtgc ctgacactgt aacactgtaa ggattgttcc aaatactagt tgcactgctc 660
tgtttataat tgtaaatatt agacaaacag tagacaaatg cagcagcaag tcaattgtat 720
tagcagaata ttgtcctcat tgcattgtga gttgtagctc gagtcccaa ccttacggcc 780
aagtttcttc tagtatgatg gaaagtttct tttttctttg ctctgaataa aactgaactg 840
tggtttctct ataagtggca ttttgggctt tcctcccttt tttgtaaagc aatgtctgcc 900
tagtttattg tccaagttaa ctttaggtga ccttttaaaa gttggcattg aaaataaaaac 960
aacttgcaaa aaagtttctt ggaatagaat taacaaaata ttatctttat catgagttgg 1020
aaactggaaa aaggcttctt gaagtaaagt ttctgagtgg agctactagg atgtcttcca 1080
gcctcctgca gtcaaggagt accactgtat tgattagcct gtatgtagca gggctccctt 1140
cattgcatct gaggacttgt tttctttttc tttattttta atcctcttag ttttaaatat 1200
attgcctaga gactcagtta ctaccagtt tgtggtttt tgggagaaat gtaactggac 1260
agttagcttt tcaattaaaa agacacttaa cccatgtggg atgtcatctt tttataatta 1320
gtgttcccat gtggagaaaa ttattcacac tacttgcatg taaagaataa ttttaactttt 1380
aacattaaaa tatgtggtaa aaccagaaa gcatccatca tgaatgcaag atactttcaa 1440
taaagtaagt tatat 1455

```

&lt;210&gt; 69

&lt;211&gt; 163

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 69

```

Met Pro Ser Ile Lys Leu Gln Ser Ser Asp Gly Glu Ile Phe Glu Val
  1             5             10             15

Asp Val Glu Ile Ala Lys Gln Ser Val Thr Ile Lys Thr Met Leu Glu
      20             25             30

Asp Leu Gly Met Asp Asp Glu Gly Asp Asp Asp Pro Val Pro Leu Pro
      35             40             45

Asn Val Asn Ala Ala Ile Leu Lys Lys Val Ile Gln Trp Cys Thr His
      50             55             60

His Lys Asp Asp Pro Pro Pro Pro Glu Asp Asp Glu Asn Lys Glu Lys
      65             70             75             80

Arg Thr Asp Asp Ile Pro Val Trp Asp Gln Glu Phe Leu Lys Val Asp
      85             90             95

Gln Gly Thr Leu Phe Glu Leu Ile Leu Ala Ala Asn Tyr Leu Asp Ile
      100             105             110

Lys Gly Leu Leu Asp Val Thr Cys Lys Thr Val Ala Asn Met Ile Lys
      115             120             125

Gly Lys Thr Pro Glu Glu Ile Arg Lys Thr Phe Asn Ile Lys Asn Asp
      130             135             140

Phe Thr Glu Glu Glu Glu Ala Gln Val Arg Lys Glu Asn Gln Trp Cys
      145             150             155             160

Glu Glu Lys

```

<210> 70  
<211> 2510  
<212> DNA  
<213> Homo sapiens

<400> 70  
aagatccttt ctgagctgct gtgaataaat ttggaatggt actgtatatt tccatctaatt 60  
ggagaactag ctgtactttg aataaggatt gctgcactgg acgacttttag aacatccctc 120  
acaatgtcgt caacccggag ccagaacccc cacggcctga agcagattgg cctggaccag 180  
atctgggacg acctcagagc cggcatccag cagggtgtaca cacggcagag catggccaag 240  
tccagatata tggagctcta cactcatggt tataactact gtactagtgt tcaccagttt 300  
gttggcctgg aattatataa acgacttaag gaatttttga agaattactt gacaaatctt 360  
cttaaggatg gagaagattt gatggatgag agtgtactga aattctacac tcaacaatgg 420  
gaagattatc gattttcaag caaagtgtcg aatggaattt gtgcctacct caatagacat 480  
tgggttcgcc gtgaatgtga cgaaggacga aaaggaaat atgaaatcta ttcgcttgca 540  
ttggtgactt ggagagactg tctgttcagg ccactgaata aacaggtaac aaatgctgtt 600  
ttaaagctga ttgaaaagga aaggaaatggt gaaaccatca atacaagatt gattagtggg 660  
gttgtacagt cttacgtgga attggggctg aatgaagatg atgcatttgc aaagggccct 720  
acgttaacag tgtataaaga atcctttgaa tctcaatttt tggctgacac agagagattt 780  
tataccagag agagtactga attcttgcag cagaaccag ttactgaata tatgaaaaag 840  
gcagaggctc gtctgcttga ggaacaacga agagtccagg ttaccttca tgaaagcaca 900  
caagatgaat tagcaaggaa atgtgaacaa gtcctcattg aaaaacactt ggaaattttc 960  
cacacagaat ttcagaattt attggatgct gacaaaaatg aagatttggg acgcatgtat 1020  
aatcttgtat ctagaatcca ggatggccta ggagaattga aaaaactggt ggagacacac 1080  
attcataatc agggctcttg agccattgaa aagtgtggag aagctgcttt aaatgacccc 1140  
aaaatgtatg tacagacagt gcttgatggt cataaaaaat acaatgccct ggtaatgtct 1200  
gcattcaaca atgacgctgg ctttgtggct gctcttgata aggcttgtgg tcgcttcata 1260  
aacaacaacg cggttaccaa gatggcccaa tcatccagta aatcccctga gttgctggct 1320  
cgatactgtg actccttgtt gaagaaaagt tccaagaacc cagaggaggc agaactagaa 1380  
gacacactca atcaagtgt gggtgtcttc aagtacatag aagacaaaaga cgtatttcag 1440  
aagtcttatg cgaagatgct cgccaagagg ctctgccacc agaacagtgc aagtgcgat 1500  
gccgaagcca gcatgatctc caagttaaag caagcttgcg gggttcgagta cacctctaaa 1560  
ccttgacaaa actcagaacc cctagacttg gatttcagca ttcaagtgtc gagctccggg 1620  
tccctggcct tccagcagtc ttgtacattt gccttgccgt cagagttgga acgtagtatt 1740  
cagcgattca cagctttcta cgccagccgc cacagtggcc gaaaattgac gtgggttatat 1800  
cagttgtcta aaggagaatt ggtaactaac tgcttcaaaa acagatatac tttgcaggcg 1860  
tcgacattcc agatggctat cctgcttcag tacaacacgg aagatgccta cactgtgcag 1920  
cagctgaccg acagcactca aattaaaaatg gacattttgg cgcaagtttt acagatttta 1980  
ttaaagtcga agctattggg cttggaagat gaaaatgcaa atgttgatga ggtggaattg 2040  
aagccagata ccttaataaaa attatatctt ggttataaaa ataagaaatt aaggggtaac 2100  
atcaatgtgc caatgaaaac cgaacagaag caggaacaag aaaccacaca caaaaacatc 2160  
gaggaagacc gcaaactact gattcaggcg gccatcgtga gaatcatgaa gatgaggaag 2220  
gttctgaaac accagcagtt acttggcgag gtcctcactc agctgtcctc caggttcaaa 2280  
cctcgagtcc ctgtgatcaa gaaatgcatt gacattctaa ttgagaaaga atatttggag 2340  
cgagtggatg gtgaaaagga cacctacagt tacttggctt aacccttctg gaagggctctg 2400  
actgtgtgac ccgcagcaaa tagttcatgt tggaaagaat gaaaacaact tcaagttcat 2460  
aggcagccag cctgccgcca ttggacctcc cttttaaaaa ctgaggacca 2510

<210> 71  
<211> 752  
<212> PRT  
<213> Homo sapiens

<400> 71  
Met Ser Ser Thr Arg Ser Gln Asn Pro His Gly Leu Lys Gln Ile Gly  
1 5 10 15

72

Leu Asp Gln Ile Trp Asp Asp Leu Arg Ala Gly Ile Gln Gln Val Tyr  
 20 25 30  
 Thr Arg Gln Ser Met Ala Lys Ser Arg Tyr Met Glu Leu Tyr Thr His  
 35 40 45  
 Val Tyr Asn Tyr Cys Thr Ser Val His Gln Phe Val Gly Leu Glu Leu  
 50 55 60  
 Tyr Lys Arg Leu Lys Glu Phe Leu Lys Asn Tyr Leu Thr Asn Leu Leu  
 65 70 75 80  
 Lys Asp Gly Glu Asp Leu Met Asp Glu Ser Val Leu Lys Phe Tyr Thr  
 85 90 95  
 Gln Gln Trp Glu Asp Tyr Arg Phe Ser Ser Lys Val Leu Asn Gly Ile  
 100 105 110  
 Cys Ala Tyr Leu Asn Arg His Trp Val Arg Arg Glu Cys Asp Glu Gly  
 115 120 125  
 Arg Lys Gly Ile Tyr Glu Ile Tyr Ser Leu Ala Leu Val Thr Trp Arg  
 130 135 140  
 Asp Cys Leu Phe Arg Pro Leu Asn Lys Gln Val Thr Asn Ala Val Leu  
 145 150 155 160  
 Lys Leu Ile Glu Lys Glu Arg Asn Gly Glu Thr Ile Asn Thr Arg Leu  
 165 170 175  
 Ile Ser Gly Val Val Gln Ser Tyr Val Glu Leu Gly Leu Asn Glu Asp  
 180 185 190  
 Asp Ala Phe Ala Lys Gly Pro Thr Leu Thr Val Tyr Lys Glu Ser Phe  
 195 200 205  
 Glu Ser Gln Phe Leu Ala Asp Thr Glu Arg Phe Tyr Thr Arg Glu Ser  
 210 215 220  
 Thr Glu Phe Leu Gln Gln Asn Pro Val Thr Glu Tyr Met Lys Lys Ala  
 225 230 235 240  
 Glu Ala Arg Leu Leu Glu Glu Gln Arg Arg Val Gln Val Tyr Leu His  
 245 250 255  
 Glu Ser Thr Gln Asp Glu Leu Ala Arg Lys Cys Glu Gln Val Leu Ile  
 260 265 270  
 Glu Lys His Leu Glu Ile Phe His Thr Glu Phe Gln Asn Leu Leu Asp  
 275 280 285  
 Ala Asp Lys Asn Glu Asp Leu Gly Arg Met Tyr Asn Leu Val Ser Arg  
 290 295 300  
 Ile Gln Asp Gly Leu Gly Glu Leu Lys Lys Leu Leu Glu Thr His Ile  
 305 310 315 320  
 His Asn Gln Gly Leu Ala Ala Ile Glu Lys Cys Gly Glu Ala Ala Leu

325	330	335
Asn Asp Pro Lys Met Tyr Val Gln Thr Val Leu Asp Val His Lys Lys		
340	345	350
Tyr Asn Ala Leu Val Met Ser Ala Phe Asn Asn Asp Ala Gly Phe Val		
355	360	365
Ala Ala Leu Asp Lys Ala Cys Gly Arg Phe Ile Asn Asn Asn Ala Val		
370	375	380
Thr Lys Met Ala Gln Ser Ser Ser Lys Ser Pro Glu Leu Leu Ala Arg		
385	390	395
Tyr Cys Asp Ser Leu Leu Lys Lys Ser Ser Lys Asn Pro Glu Glu Ala		
405	410	415
Glu Leu Glu Asp Thr Leu Asn Gln Val Met Val Val Phe Lys Tyr Ile		
420	425	430
Glu Asp Lys Asp Val Phe Gln Lys Phe Tyr Ala Lys Met Leu Ala Lys		
435	440	445
Arg Leu Val His Gln Asn Ser Ala Ser Asp Asp Ala Glu Ala Ser Met		
450	455	460
Ile Ser Lys Leu Lys Gln Ala Cys Gly Phe Glu Tyr Thr Ser Lys Leu		
465	470	475
Gln Arg Met Phe Gln Asp Ile Gly Val Ser Lys Asp Leu Asn Glu Gln		
485	490	495
Phe Lys Lys His Leu Thr Asn Ser Glu Pro Leu Asp Leu Asp Phe Ser		
500	505	510
Ile Gln Val Leu Ser Ser Gly Ser Trp Pro Phe Gln Gln Ser Cys Thr		
515	520	525
Phe Ala Leu Pro Ser Glu Leu Glu Arg Ser Tyr Gln Arg Phe Thr Ala		
530	535	540
Phe Tyr Ala Ser Arg His Ser Gly Arg Lys Leu Thr Trp Leu Tyr Gln		
545	550	555
Leu Ser Lys Gly Glu Leu Val Thr Asn Cys Phe Lys Asn Arg Tyr Thr		
565	570	575
Leu Gln Ala Ser Thr Phe Gln Met Ala Ile Leu Leu Gln Tyr Asn Thr		
580	585	590
Glu Asp Ala Tyr Thr Val Gln Gln Leu Thr Asp Ser Thr Gln Ile Lys		
595	600	605
Met Asp Ile Leu Ala Gln Val Leu Gln Ile Leu Leu Lys Ser Lys Leu		
610	615	620
Leu Val Leu Glu Asp Glu Asn Ala Asn Val Asp Glu Val Glu Leu Lys		
625	630	635
		640



Pro Asp Thr Leu Ile Lys Leu Tyr Leu Gly Tyr Lys Asn Lys Lys Leu  
645 650 655

Arg Val Asn Ile Asn Val Pro Met Lys Thr Glu Gln Lys Gln Glu Gln  
660 665 670

Glu Thr Thr His Lys Asn Ile Glu Glu Asp Arg Lys Leu Leu Ile Gln  
675 680 685

Ala Ala Ile Val Arg Ile Met Lys Met Arg Lys Val Leu Lys His Gln  
690 695 700

Gln Leu Leu Gly Glu Val Leu Thr Gln Leu Ser Ser Arg Phe Lys Pro  
705 710 715 720

Arg Val Pro Val Ile Lys Lys Cys Ile Asp Ile Leu Ile Glu Lys Glu  
725 730 735

Tyr Leu Glu Arg Val Asp Gly Glu Lys Asp Thr Tyr Ser Tyr Leu Ala  
740 745 750

<210> 72  
<211> 1015  
<212> DNA  
<213> Homo sapiens

<400> 72  
cctagcctcg cctccggttac aacagcctac ggtgctggag gatccttctg cgcacgcgca 60  
cagcctccgg ccggctatatt ccgcgagcgc gttccatcct ctaccgagcg cgcgcgaaga 120  
ctacggaggt cgactcggga gcgcgcacgc agctccgccc cgcgtccgac ccgcggatcc 180  
cgcggcgtcc ggcccgggtg gtctggatcg cggagggaat gcccggagg gcggagaact 240  
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## INTERNATIONAL SEARCH REPORT

Int l Application No

PCT/US 00/15449

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Minimum documentation searched (classification system followed by classification symbols)

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MEDLINE, CHEM ABS Data, WPI Data, PAJ, BIOSIS, EPO-Internal, STRAND

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 11176 A (BEACH DAVID ;ZHANG HUI (US); COLD SPRING HARBOR LAB (US)) 27 March 1997 (1997-03-27) the whole document	1,2,6,7, 9,14-20, 27
X	YU Z K ET AL: "Human CUL-1 associates with the SKP1/ SKP2 complex and regulates p21(CIP1/WAF1) and cyclin D proteins." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 SEP 15) 95 (19) 11324-9. , XP002151555 cited in the application See especially page 11328, discussion the whole document	1,2,6,7, 9,14-19

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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European Patent Office, P.B. 5618 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.  
Fax: (+31-70) 340-3016

Authorized officer

Groenendijk, M

## INTERNATIONAL SEARCH REPORT

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	WO 99 18989 A (BAYLOR COLLEGE MEDICINE) 22 April 1999 (1999-04-22) the whole document	1-5
X	HATAKEYAMA E.A.: "Ubiquitin-dependent degradation of IkBa is mediated by a ubiquitin ligase Skp1/Cul-1/F-box protein FWD1" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 96, March 1999 (1999-03), pages 3859-3863, XP002152297 WASHINGTON US the whole document	1,3
X	ZHANG H ET AL: "p19Skp1 and p45Skp2 are essential elements of the cyclin A-CDK2 S phase kinase." CELL, (1995 SEP 22) 82 (6) 915-25. , XP002151557 cited in the application The whole document; see especially page 920, column 2	14-19
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## INTERNATIONAL SEARCH REPORT

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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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P,X	WINSTON E.A.: "A family of mammalian F-box proteins" CURRENT BIOLOGY, vol. 9, 11 October 1999 (1999-10-11), pages 1180-1192, XP000960309 the whole document	1-5
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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/15449

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9711176	A	27-03-1997	US	5981702 A	09-11-1999
			CA	2230138 A	27-03-1997
WO 9918989	A	22-04-1999	AU	1088399 A	03-05-1999

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(71) Applicant (for all designated States except US): YALE  
UNIVERSITY [US/US]; 451 College Street, New Haven,  
CT 06520 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): ZHANG, Hui  
[US/US]; 203 Schoolside Lane, Guilford, CT 06437 (US).  
TSVETKOV, Lyuben, M. [BG/US]; 137 Cottage Street,  
Apt. E3, New Haven, CT 06517 (US). KONDO, Takeshi  
[JP/US]; 115 Florence Road, Apt. 1A, Branford, CT  
06405 (US).

(74) Agent: MORGAN, LEWIS & BOCKIUS LLP; 1800 M  
Street, NW, Washington, DC 20036 (US).

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(54) Title: MODULATION OF PROTEIN LEVELS USING THE SCF COMPLEX

(57) Abstract: This invention encompasses various methods of modulating protein levels using the SKP1, CDC53/Cullin, F-box(SCF) protein complex. More specifically, the present invention provides various methods of target protein degradation using targeted ubiquitination techniques. The present invention also provides various compositions and assays associated with the disclosed modulation of protein levels using the SCF complex as well as various methods of detecting, monitoring and treating cancerous cells.

WO 00/75184 A1

## MODULATION OF PROTEIN LEVELS USING THE SCF COMPLEX

### INVENTORS

Hui Zhang, Lyuben M. Tsvetkov and Takeshi Kondo

5

### RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application No. 60/137,494 filed June 4, 1999 which is herein incorporated by reference in its entirety.

### 10 FIELD OF THE INVENTION

The present invention pertains, in general, to the field of protein knockout technology. In particular, the present invention pertains to protein knockout technology using targeted ubiquitination techniques.

### 15 BACKGROUND OF THE INVENTION

All publications, patents and patent applications discussed herein are incorporated by reference to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference. The paper by Tsvetkov *et al.*, (1999) Current Biology 9, 661-664 including S1-S2 is fully  
20 and completely herein incorporated by reference.

#### Cyclin-Dependent Kinases (CDKs)

Entry into each phase of the eukaryotic cell division cycle is regulated by proteins known as cyclin-dependent kinases (CDKs). In mammalian cells, seven different CDK  
25 protein subtypes have been described, each of which has been associated with particular phases of the cell cycle (Matsushime *et al.*, (1992) Cell 71, 323-334; Xiong *et al.*, (1992) Cell 71, 505-514; Meyerson *et al.*, (1992) EMBO J. 11, 2909-2917; Fisher & Morgan, (1994) Cell 78, 713-724; Meyerson & Harlow, (1994) Mol. Cell. Biol. 14, 2077-2086). Activation of each CDK in the cell cycle is regulated by its association with an equally  
30 diverse family of regulatory subunits known as cyclins. Multiple cyclin-CDK associations have been implicated in cell cycle control during cell proliferation in mammals. For



example, cyclin D-CDK4 is associated with cell cycle progression through G<sub>1</sub> phase while both cyclin E-CDK2 and cyclin A-CDK2 facilitate G<sub>1</sub> to S phase transition (Nasmyth & Hunt, (1993) Nature 366, 634-635).

CDKs are regulated at several different levels including phosphorylation and  
5 interaction with other proteins. Activation of CDKs is initially dependent on complex formation with their cognate cyclin subunits and is regulated at this stage by fluctuations in the levels of these subunits (Sherr, (1993) Cell 73, 1059-1065). Phosphorylation of a conserved threonine residue in CDK is essential for activation following cyclin-CDK complex formation (Solomon *et al.*, (1993) EMBO J. 12, 3133-3142; Makela *et al.*, (1994)  
10 Nature 371, 254-257). Studies have also focused on the role of CDK inhibitor proteins such as p16, p21 or p27, which act as another level of cell cycle regulation by preventing unscheduled entry into another phase of the cell cycle (Hunter & Pines, (1994) Cell 79, 573-583; Sherr, (1996) Science 274, 1672-1677). These proteins interact with specific domains surrounding the phosphorylated threonine residue on the CDK. p27 for example,  
15 inhibits cyclin E-CDK2 and has been characterized in detail (Polyak *et al.*, (1994) Cell 78 59-36; U.S. Patent No. 5,688,665).

Transformed cells differ from normal cells in their ability to proliferate indicating that alterations in pathways which control cell cycle progression accompany cellular transformation. Alterations in the regulatory events underlying cellular proliferation  
20 pathways can translate into changes in the cyclin-CDK pathways controlling cell cycle progression and has long been implicated in cellular transformation. In normal cells each cyclin-CDK complex exists in a quaternary complex that also contains proliferating cell nuclear antigen (PCNA) and a CDK inhibitor protein. These quaternary complexes are absent in transformed cells because the CDK inhibitory protein is not expressed (Zhang *et al.*, (1993) Mol. Biol. Cell 4, 897-906).  
25

For example, studies in normal human fibroblasts demonstrated that cyclin A-CDK2 was associated with p21 and PCNA in a quaternary complex. p21 and PCNA were absent in other transformed cells or established tumor cell lines, and cyclin A-CDK2 was bound to three novel proteins to form a protein complex (Zhang *et al.*, (1993) Mol.  
30 Biol. Cell 4, 897-906). The first two proteins in this complex were S-phase kinase associated proteins designated SKP1 and SKP2. The third protein, designated CUL1, is a

member of the cullin/CDC53 family of proteins. The cyclin A-CDK2/SKP1/SKP2/CUL1 complex functions as a conserved ubiquitin E3 enzyme that regulates mammalian G<sub>1</sub> to S phase transition by specifically targeting mammalian G<sub>1</sub> regulators, such as p21 for ubiquitin-dependent degradation (Yu *et al.*, (1998) Proc. Natl. Acad. Sci. USA 95, 11324-11329). Decreased levels of p21 in tumor cells confirm that p21 is being targeted for ubiquitin-dependent degradation in transformed cells (Xiong *et al.*, (1993) Genes Dev. 7, 1572-1583; Yu *et al.*, (1998) Proc. Natl Acad. Sci. USA 95, 11324-11329).

#### Ubiquitin-Dependent Protein Degradation

Ubiquitin-dependent protein degradation functions to regulate protein turnover in a cell by closely regulating the degradation of specific proteins. Once a protein is tagged with ubiquitin it is degraded in an ATP-dependent reaction by the 26S proteasome. Ubiquitin is a small protein composed of seventy-six amino acids that serves only as a tag to mark proteins for degradation. Three distinct enzymes are required for protein ubiquitination (King *et al.*, (1996) Science 274, 1652-1659). First, ubiquitin is activated in an ATP dependent reaction by forming a thioester bond with the ubiquitin activation enzyme designated E1. The activated ubiquitin is then transferred from E1 to the ubiquitin conjugating enzyme designated E2. This enzyme mediates the transfer of ubiquitin to protein substrates in conjunction with a ligase enzyme designated E3. The ubiquitinated protein substrates are then degraded by the 26S proteasome.

#### S-Phase Kinase Associated Proteins

In many DNA viral oncoprotein transformed or other established tumor cells that are deficient in p53 expression, p21 and proliferating cell nuclear antigen (PCNA) disappeared and cyclin A/CDK2 was prominently complexed with two novel proteins, S-phase kinase associated proteins 1 and 2 (SKP1 and SKP2, also known as p19 and p45, respectively) (Yu *et al.*, (1998) Proc. Natl. Acad. Sci. USA 95, 11324-11329; Zhang *et al.*, (1997) WO9711176). SKP1 and SKP2 have been isolated and the genes encoding these proteins have been sequenced (Zhang *et al.*, (1997) WO9711176; which is herein incorporated by reference in its entirety). SKP2 expression has been shown to be highly

induced in many transformed cells (Zhang *et al.*, (1995) Cell 82, 915-925, which is herein incorporated by reference in its entirety).

The SKP1/SKP2/CUL1 E3 ligase complex has been implicated in the ubiquitin-dependent degradation of p21 during cell cycle progression. Furthermore, p27  
5 has also been shown to be a target of ubiquitin-dependent degradation in a CDC34-dependent proteolytic process. CDC34 serves as a ubiquitin E2 conjugating enzyme for SCF (SKP1, CDC53/Cullin, F-box protein) complexes (Yu *et al.*, (1998) Proc. Natl. Acad. Sci. USA 95, 11324-11329; Pagano *et al.*, (1995) Science 269, 682-685; King *et al.*, (1996) Science 274, 1652-1659). The ubiquitin-dependent p27 degradation occurs during  
10 the transition from G1 to S phase as indicated by the increase in the level of SKP2 in late G1 which corresponds with a decrease in p27 levels. p27 ubiquitin-dependent degradation is also dependent on cyclin E/CDK2 activity (Brandeis & Hunt, (1996) EMBO J. 15, 5280-5289; Sheaff *et al.*, (1997) Genes Dev. 11, 1464-1478).

## 15 SUMMARY OF THE INVENTION

The present invention encompasses a method of altering the level of polypeptide in a cell comprising altering the amount of one or more of the proteins selected from the group consisting of SKP1, SKP2, SKP2-like protein and CUL-1. In a preferred  
20 embodiment, the polypeptide is phosphorylated and the SKP2-like protein is selected from the group consisting of ZF1 (SEQ ID NO: 27), ZF3 (SEQ ID NO: 29), ZF4 (SEQ ID NO: 31), ZF5 (SEQ ID NO: 33), ZF6 (SEQ ID NO: 35), ZF7 (SEQ ID NO: 37), ZF8 (SEQ ID NO: 39), ZF9 (SEQ ID NO: 41), ZF11 (SEQ ID NO: 43), ZF13 (SEQ ID NO: 45), ZF16 (SEQ ID NO: 47), ZF18 (SEQ ID NO: 49), ZF19 (SEQ ID NO: 51), ZF20 (SEQ ID NO: 53), ZF23 (SEQ ID NO: 55), ZF24 (SEQ ID NO: 57), ZF25 (SEQ ID NO: 59) and ZF26  
25 (SEQ ID NO: 61).

In yet another preferred embodiment the polypeptide in the method of the invention is selected from the group consisting of p27 (SEQ ID NO: 65), cyclin E (SEQ ID NO: 63), Max (SEQ ID NO: 9), Mad (SEQ ID NO: 11), c-Myc (SEQ ID NO: 13), MDM2 (SEQ ID NO: 15), p53 (SEQ ID NO: 17), Bax (SEQ ID NO: 19), Bad (SEQ ID  
30 NO: 21) and Bcl-2 (SEQ ID NO: 23). The method of invention may be used to increase

the level of polypeptide by decreasing the amount of SKP2 or in the alternative the level of polypeptide is reduced by increasing the amount of SKP2.

In a yet another embodiment, the invention includes a method of altering the level of SKP2 comprising altering the amount of p27 polypeptide which is available for binding with SKP2. In a further embodiment, the invention includes a method of modulating the activity of SKP2 comprising contacting SKP2 with a peptide comprising a SKP2 interaction domain which is available for binding with SKP2. In a preferred embodiment, the peptide is phosphorylated and the SKP2 interaction domain is derived from p27 or cyclin E. In a preferred embodiment, the peptide comprises any one of the amino acid sequences of SEQ ID NO: 1, 2, 3, 4, 5 or 6.

The invention also includes a method of treating a tumor in a mammal comprising altering the level of SKP protein in the cells of said tumor. In a preferred embodiment the SKP protein is SKP2 or allelic variants thereof. In a related embodiment the invention includes a method of detecting a tumor in a mammal wherein the level of SKP2 is used as a diagnostic and prognostic indicator to determine the progression of said tumor. In a preferred embodiment, the invention encompasses a method of monitoring the treatment of a tumor in a mammal wherein the level of SKP2 is used as a diagnostic and prognostic indicator.

The invention also includes methods of testing an agent for the ability to modulate an interaction between SKP2 and a target protein wherein the method comprises (a) fusing SKP2 with a target protein interaction domain to produce a SKP2 fusion protein; (b) contacting the agent, the SKP2 fusion protein and the target protein; and (c) determining whether the interaction of the SKP2 fusion protein with the target protein has been modulated by the agent.

The invention further encompasses a method of altering the level of a target protein in a cell comprising inserting a heterologous target protein interaction domain with SKP2 or a SKP2-like protein to produce a fusion protein, and contacting fusion protein with the target protein. In a preferred embodiment, the SKP-2 like protein is selected from the group consisting of ZF1 (SEQ ID NO: 27), ZF3 (SEQ ID NO: 29), ZF4 (SEQ ID NO: 31), ZF5 (SEQ ID NO: 33), ZF6 (SEQ ID NO: 35), ZF7 (SEQ ID NO: 37), ZF8 (SEQ ID NO: 39), ZF9 (SEQ ID NO: 41), ZF11 (SEQ ID NO: 43), ZF13 (SEQ ID NO: 45), ZF16 (SEQ

ID NO: 47), ZF18 (SEQ ID NO: 49), ZF19 (SEQ ID NO: 51), ZF20 (SEQ ID NO: 53), ZF23 (SEQ ID NO: 55), ZF24 (SEQ ID NO: 57), ZF25 (SEQ ID NO: 59) and ZF26 (SEQ ID NO: 61).

5 In yet another embodiment, the invention includes a method of altering the level of a target protein in a cell comprising expressing a cDNA coding for a SKP2 fusion protein comprising a SKP2 protein fused with a target protein interaction domain which is specific for the target protein. In a related embodiment, the invention includes a method of ubiquitinating a target protein comprising fusing a target protein interaction domain with SKP2, and contacting the SKP2 fusion protein with the target protein. In preferred  
10 embodiments, the target protein is selected from the group consisting of p27 (SEQ ID NO: 65), cyclin E (SEQ ID NO: 63), Max (SEQ ID NO: 9), Mad (SEQ ID NO: 11), c-Myc (SEQ ID NO: 13), MDM2 (SEQ ID NO: 15), p53 (SEQ ID NO: 17), Bax (SEQ ID NO: 19), Bad (SEQ ID NO: 21) and Bcl-2 (SEQ ID NO: 23).

The invention also includes a method of modulating protein ubiquitination  
15 comprising altering the amount of SKP2 which is available to facilitate protein ubiquitination.

Finally, the invention encompasses a fusion protein comprising a first protein comprising at least one SKP2 C-terminal motif (SCM) capable of interacting with SKP1 and forming a complex with CUL-1 and a second protein which is capable of interacting  
20 with a heterologous target protein. In a preferred embodiment, the fusion protein contains only one SCM capable of interacting with SKP1. In another preferred embodiment, the SCM is selected from any one of the following proteins selected from the group consisting of SKP2 (SEQ ID NO: 67), ZF1 (SEQ ID NO: 27), ZF3 (SEQ ID NO: 29), ZF4 (SEQ ID NO: 31), ZF5 (SEQ ID NO: 33), ZF6 (SEQ ID NO: 35), ZF7 (SEQ ID NO: 37), ZF8 (SEQ  
25 ID NO: 39), ZF9 (SEQ ID NO: 41), ZF11 (SEQ ID NO: 43), ZF13 (SEQ ID NO: 45), ZF16 (SEQ ID NO: 47), ZF18 (SEQ ID NO: 49), ZF19 (SEQ ID NO: 51), ZF20 (SEQ ID NO: 53), ZF23 (SEQ ID NO: 55), ZF24 (SEQ ID NO: 57), ZF25 (SEQ ID NO: 59) and ZF26 (SEQ ID NO: 61).

30

## BRIEF DESCRIPTION OF THE DRAWINGS

### Figure 1 - Phosphorylation-dependent p27 degradation in HeLa extracts

(A) *In vitro* translated,  $^{35}\text{S}$ -labeled p27 or p27 T187G mutant was incubated with HeLa extracts for 3 hours at 30°C. The addition of cyclin E/CDK2 and the 26S proteasome inhibitor, MG132 (20  $\mu\text{l}$ ) is indicated. The p27 reaction products were isolated by immunoprecipitation and visualized by autoradiography. (B) Time course of p27 degradation using baculovirus produced and  $^{35}\text{S}$ -labeled p27 (0.5  $\mu\text{g}$ ). (C) Baculovirus-expressed,  $^{35}\text{S}$ -labeled p27 was incubated with HeLa extracts in the absence or presence of cyclin E/CDK2 and MG132. The reaction products were treated with lambda phosphatase (PPTase). The phosphorylated and high molecular weight p27 species (in brackets) are indicated.

### Figure 2 - Ubiquitination of p27 in the HeLa cytosolic extracts

Accumulation of ubiquitinated p27 in the presence of modified ubiquitins. p27 was incubated with HeLa extracts, cyclin F/CDK2, and methyl ubiquitin (UbM, 0.5 mg/ml) and ubiquitin aldehyde (UbA, 1  $\mu\text{M}$ ) as indicated. The ubiquitinated p27 ladders accumulated because methylated ubiquitin shortens the polyubiquitinated chain and thus slows down the rate of degradation while ubiquitin aldehyde inhibits de-ubiquitination of ubiquitinated proteins by isopeptidases.

20

### Figure 3 - Inhibition of p27 degradation by depletion of the SCF<sup>SKP2</sup> complex

(A) Depletion of CUL-1 abolishes p27 degradation. HeLa extracts were passed through the affinity purified CUL-1 antibody or a control IgG column. The control and CUL-1 depleted extracts were assayed for p27 degradation activity at various times at 30°C in the presence of cyclin E/CDK2. The left three lanes are p27 input and regular HeLa extracts and incubated for three hours. Reaction products were treated with lambda phosphatase. (B) SKP1 depleted extracts. The extracts were control depleted using IgG or depleted with an SKP1 antibody column and then incubated with p27 as described in A. The reaction products were not treated with the phosphatase so the phosphorylated p27 are shown. Ext: regular HeLa extracts. (C) SKP2 depleted extracts. SKP2 was immuno-depleted as described in A and B. The control and SKP2 depleted extracts were incubated for three

hours at 30 °C and the reaction products were analyzed after phosphatase treatment. (D) Specific removal of SKP2, SKP1, and CUL-1 by the immuno-depletion processes. The regular HeLa (Ext), control depleted, and SKP2, SKP1 or CUL-1 depleted extracts were Western-blotted by SKP2 (top), SKP1 (middle), and CUL-1 (bottom) antibodies, respectively.

Figure 4 - SCF<sup>SKP2</sup> complex specific interactions with thr187 phosphorylated p27 peptide

(A) Sequences of p27 carboxy-terminal peptides (amino acids 175-198) with or without threonine 187 phosphorylation. (B) SKP2 specifically binds to the threonine 187 phosphopeptide of p27. F-box proteins, SKP2,  $\beta$ -TrCP, and MD6 were *in vitro* translated as <sup>35</sup>S-labeled proteins. The proteins (10  $\mu$ l each) were incubated for one hour with the p27 peptide or threonine 187 phosphopeptide beads. The proteins associated with the beads were purified and analyzed. (C) Selective binding of SKP2, SKP1 and CUL-1 in the HeLa extracts to the p27 threonine 187 phosphopeptide. HeLa extracts (400  $\mu$ g) were incubated with the p27 phosphopeptide or peptide beads for one hour. The proteins associated with the beads were analyzed by Western-blot analysis with either SKP2, SKP1, CUL-1 or  $\beta$ -TrCP antibodies, as indicated. The immunoprecipitated SKP2, SKP1 and CUL-1 are included as a control as indicated. For lane four,  $\beta$ -TrCP, HeLa extracts (25  $\mu$ g) were directly loaded without immunoprecipitation. HeLa extracts for SKP2 immunoprecipitation were 25  $\mu$ g while for SKP1 and CUL-1 were 100  $\mu$ g. (D) Association between SKP1, SKP2 and CUL-1 in the HeLa extracts. The SKP1, SKP2 and CUL-1 were immunoprecipitated by specific antibodies, respectively, as indicated. The immunoprecipitated proteins were examined for the presence of SKP2 by Western-blotting with SKP2 antibodies. (E) Association between SKP1 and p27 phosphopeptide depends on the presence of SKP2. HeLa (Ext), Mock or SKP2 depleted extracts (100  $\mu$ g each) were incubated either with p27 peptide (pept) or phosphopeptide (phophopept) beads. The proteins associated with the peptide beads were examined for the presence of SKP2 or SKP1 by Western blot. SKP2 and SKP1 in the mock and SKP2 depleted extracts (25  $\mu$ g each) were also examined by direct Western blotting of the extracts. Depl: depletion of extracts by pre-immune IgG (Mock) or SKP2 antibodies.

Figure 5 - SCF<sup>SKP2</sup> complex contains a p27 ubiquitination E3 activity

- (A) Restoration of p27 degradation activity in SKP2 depleted extracts by recombinant SCF<sup>SKP2</sup>. Insect SF9 cells were co-infected with baculoviruses encoding GST-SKP1 and CUL-1, either in the presence (SCF<sup>SKP2</sup>) or in the absence of SKP2 (SC) baculoviruses.
- 5 The SCF<sup>SKP2</sup> and SC complexes were isolated by glutathione Sepharose. The recombinant SCF<sup>SKP2</sup>, SC (one µg each in two µl), or the buffer (two µl) was added into the SKP2 depleted extracts (200 µg), as indicated, and p27 degradation was assayed in the presence of cyclin E/CDK2. HeLa extract (ext) was included as the control. (B) p27 ubiquitination using recombinant proteins. p27 was incubated with the recombinant SCF<sup>SKP2</sup> complex, in
- 10 the presence of purified cyclin E and cyclin A/CDK2 kinases, human E1 ubiquitin activating enzyme, ATP, ubiquitin, and recombinant CDC34, an E2 ubiquitin conjugation enzyme (lane 2). The reactions in lanes 3 and 4 were conducted in the absence of either ubiquitin or CDC34, respectively.
- 15 Figure 6 - SKP2 specifically binds to the phosphorylated Thr380 in cyclin E
- (A) Sequences of the cyclin E carboxy-terminal peptides (residues 371-394) with (TP-CP) or without Thr380 (TP-C) phosphorylation. (B) SKP2 is specific for cyclin E phosphopeptide binding. *In vitro*-translated and <sup>35</sup>S-labeled F-box proteins SKP2, FBL2, 5, 6, 7, and 8 (10 µl each) were incubated with the TP-C or TP-CP beads. Their
- 20 associations with cyclin E peptide beads were analyzed. Lysates: translated lysate control. (C) Specific interaction between SKP2 and the Thr380-phosphorylated cyclin E peptide in HeLa cell extracts. 400 µg of HeLa cytosolic extracts were incubated with either TP-C or TP-CP beads (25 µl) for one hour. The proteins associated with the beads were Western-blotted with antibodies against SKP2, SKP1, or CUL-1. Left lane, HeLa lysate control.
- 25 (D) Upper panel - The phosphorylated TP motif is required for specific SKP2 interaction. Mutant derivatives of cyclin E peptides were synthesized in which either Thr380 is converted into serine or phosphoserine (SP-C or SP-CP) or Pro381 is converted into alanine in TP-CP (TA-CP). The beads bearing the wild-type and the mutant cyclin E peptides were assayed for SKP2 binding as described in (C). Lower panel - competition of
- 30 the interaction between SKP2 and cyclin E Thr380 peptide beads (TP-CP) with wild-type or mutant cyclin E phosphopeptides. 25 µl TP-CP beads were incubated with HeLa



extracts (400 µg) in the absence or in the presence of either TP-CP (10, 50 or 250 µg/ml) or equal amounts of TA-CP or SP-CP. The association between SKP2 and the TP-CP beads was analyzed by Western-blot.

5 **Figure 7 - SKP2 promotes cyclin E ubiquitination and degradation**

(A) Dependency of SKP2-mediated cyclin E degradation on Thr380 in cyclin E. T7-cyclin E or cyclin E T380G mutant constructs were transfected into HeLa cells in the presence or absence of SKP2 expression vector. Cell lysates were prepared in an SDS-containing buffer and 40 µg of each lysate were loaded directly onto a protein gel. The  
10 proteins were detected by Western-blotting with anti-T7 (top), SKP2 (middle), and CDK2 (lower) antibodies. (B) SKP2-induced formation of high molecular-weight species of cyclin E is sensitive to the Thr380 mutation in cyclin E. One microgram of T7-cyclin E or cyclin E T380G mutant expression constructs was transfected into 293 cells in the absence or presence of increasing amounts of the SKP2 construct (0, 0.25, 0.5, 1 and 2.5 µg,  
15 respectively). The proteins were detected by anti-T7 (top) or SKP2 (lower) antibodies. (C) SKP2 and ubiquitin both induce high-molecular-weight species of cyclin E. Expression vectors encoding SKP2 (5 µg), T7-tagged cyclin E (1 µg), or HA-tagged ubiquitin (HAUb, 1 µg) were transfected into 293 cells as indicated. Twenty-four hours post-transfection, cells were treated with LLNL for six hours. The proteins were detected  
20 by either anti-T7 monoclonal (top and middle panels) or anti-SKP2 (lower panel) antibodies. The middle panel is a lighter exposure of the top panel. (D) SKP2 promotes polyubiquitination of cyclin E. Expression vectors encoding SKP2 (5 µg), T7-tagged cyclin E (1 µg), or HA-tagged ubiquitin (HAUb, 0.1 µg) or a combination of them were transfected into 293 cells as indicated. The proteins were immunoprecipitated with the  
25 anti-HA antibody (12CA5) for ubiquitinated proteins followed by Western-blotting with anti-T7 antibody for cyclin E. (E) SKP2-mediated cyclin E ubiquitination is p27-independent but requires Thr380. p27<sup>-/-</sup> mouse embryonic fibroblasts were transfected with T7-cyclin E, SKP2 expression constructs, or both as described in B. The proteins were detected by anti-T7 (top) or SKP2 (lower) antibodies.

30

**Figure 8 - SKP2 affects cyclin E stability by directly binding to cyclin E**

(A) SKP2 shortens the half-life of the cyclin E protein. Tagged-cyclin E expression construct was transfected into HeLa cells in the absence or in the presence of SKP2. Twenty-four hours after transfection, the cells were pulse-labeled with  $^{35}\text{S}$ -methionine for thirty minutes. The labeling medium was removed and the cells were chased in fresh medium containing 1 mM unlabeled methionine. The cells were harvested at various points (0, 1, 2, 3 and 4 hours) in the chasing medium and the labeled cyclin E protein was immunoprecipitated and examined. (B) Association of cyclin E with SKP2 *in vivo*. p27<sup>-/-</sup> mouse embryonic fibroblasts were transfected with DNA expression constructs encoding LacZ ( $\beta$ -Gal), T7-cyclin E, or the T380G cyclin E mutant. The lysates were prepared and immunoprecipitated with anti-cyclin E (left) or anti-SKP2 antibodies. The presence of cyclin E in the immunoprecipitates was examined with the anti-T7 antibody by Western-blotting. (C) Cyclin E degradation is inhibited by p27. SKP2 (5  $\mu\text{g}$ ), T7-tagged cyclin E (1  $\mu\text{g}$ ), LacZ ( $\beta$ -Gal), or p27 T187G mutant (1  $\mu\text{g}$ ) expression constructs were transfected into HeLa cells as indicated. The levels of cyclin E and p27 T187G mutant were detected with T7 and p27 antibodies.

#### Figure 9 - Effects of SKP2 on endogenous cyclin E

(A) SKP2 decreases the levels of endogenous cyclin E. U87EcoR cells were infected with recombinant retroviruses encoding either LacZ ( $\beta$ -gal) or SKP2. Thirty-six or sixty hours after infection, cell lysates were prepared and 40  $\mu\text{g}$  of lysates were used for examination of the levels of endogenous cyclin E, CDK2, p27 and the expression of SKP2 by Western-blotting using their specific antibodies. (B) SKP2 induces cyclin E down-regulation in S-phase cells. Thirty-six hours post-retrovirus-infection, cells were treated with 5 mM HU for twenty-four more hours to synchronize cells in S phase. The levels of endogenous cyclin E and CDK2 as well as the expression of SKP2 were examined. (C) Expression of a dominant negative SKP2 mutant causes the accumulation of endogenous cyclin E. Glioblastoma U87EcoR cells were infected with recombinant retroviruses containing either an empty vector or a SKP2 dominant negative mutant (SKP2DN). The levels of either endogenous cyclin E, p27, SKP2 as well as the exogenous SKP2DN were examined forty-eight hours after infection. (D) The dominant negative effect of the SKP2 mutant on

cyclin E accumulation is p27-independent. The experiment was performed in essentially the same way as in C, except that p27<sup>-/-</sup> mouse embryonic fibroblasts were used.

Figure 10 - Alteration of the substrate-specificity of F-box proteins

- 5 The  $\beta$ -TRCP and SKP2 hybrid protein was generated to alter the substrate-specificity of  $\beta$ -TRCP to that of SKP2. The cDNA encoding the amino-terminus domain of  $\beta$ -TRCP (residues 1-204, including the F-box) was amplified with PCR and cloned into Bluescript at XhoI site. The cDNA containing the carboxy-terminus region of SKP2 without the F-box but retaining the LRR region (residues 169-435, without the F-box) was similarly
- 10 amplified and fused with the amino-terminal region of  $\beta$ -TRCP. The resulting cDNA encoding the TRCP.N/SKP2.C hybrid protein is cloned into pcDNA3 under CMV promoter control. The corresponding truncated SKP2 carboxy-terminal region (SKP2.C) or the amino-terminal region of  $\beta$ -TRCP ( $\beta$ -TRCP.N) was also cloned into pcDNA3.

15

Figure 11 - TRCP.N/SKP2.C hybrid induces formation of polyubiquitinated cyclin E

- One microgram of T7-tagged cyclin E were transfected into 293 human embryonic kidney cells in the presence of either the control empty vector, SKP2, SKP2 amino-terminal region (SKP2.N, residues 1-168),  $\beta$ -TRCP amino-terminal region (TRCP.N),
- 20 TRCP.N/SKP2.C hybrid, or SKP2 carboxy-terminal region (5  $\mu$ g each) by the calcium phosphate method. Both SKP2.N and TRCP.N contain the F-box. Cell lysates were prepared twenty-four hours post-transfection in an SDS-containing buffer and 40  $\mu$ g of each lysate were loaded directly onto a protein SDS-PAGE gel. The proteins were detected by Western-blotting with anti-T7 antibodies for the transfected cyclin E.

25

Figure 12 - F-box amino acid sequence alignment

Homologies within the F-box region between various F-box containing proteins.

Figure 13. - SCM domain amino acid sequence alignment

- 30 Homology between the  $\alpha$  domain of the von Hippel-Linda protein (VHL) (SEQ ID NO: 73) and the SCM domain of SKP2.

Figure 14 - Dependency of SKP2-mediated cyclin E degradation on Thr380 in cyclin E  
T7-cyclin E or cyclin E T380G mutant constructs were transfected into HeLa cells in the  
presence or absence of SKP2 expression vector. Cell lysates were prepared and 40 µg of  
5 each lysate was loaded directly onto a protein SDS PAGE gel. The proteins were detected  
by Western-blotting with anti-T7 (upper) and SKP2 (lower) antibodies.

Figure 15 - Isolated SCF<sup>SKP2</sup> complex contains ubiquitination activity  
The immunoprecipitated complex was incubated for 1 hour at 30°C with 6 µM ubiquitin, 2  
10 mM ATP, 50 mM creatine phosphate, 20 µg/ml creatine kinase, 1 µg purified ubiquitin  
activating enzyme E1, 1 µg purified E2 conjugating enzyme CDC34 in a buffer containing  
20 mM Hepes, pH 7.2, 10 mM MgCl<sub>2</sub>, 1 mM DTT. The ubiquitin reaction was terminated  
by addition of 0.5% SDS and loaded directly in an SDS-PAGE protein gel. The  
ubiquitinated proteins were detected by Western-blotting with the anti-ubiquitin antibody  
15 (Chemcon International).

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unless defined otherwise, all technical and scientific terms used herein have the  
same meaning as commonly understood by one of ordinary skill in the art to which this  
20 invention belongs. Although any methods and materials similar or equivalent to those  
described herein can be used in the practice or testing of the present invention, the  
preferred methods and materials are described.

### A. Definitions

25 As used herein, the term "agent" means any molecule that is randomly selected or  
rationally designed. As used herein, an agent is said to be randomly selected when the  
agent is chosen randomly without considering the specific sequences involved in the  
association of the proteins under study or the known functions of the proteins under study.  
An example of randomly selected agents is the use a chemical library, a peptide  
30 combinatorial library, or a growth broth of an organism.

As used herein, an agent is said to be “rationally designed” when the agent is chosen on a non-random basis which takes into account the sequence of the proteins under study and/or their conformation in connection with the agent’s action. Agents can be rationally selected or rationally designed by utilizing the amino acid sequences that make up potential contact sites between the proteins. For example, a rationally selected peptide agent can be a peptide whose amino acid sequence is identical to an identified contact site on one of the proteins under study. Such an agent will reduce or block the association of the protein with its binding partner by binding to the contact site on the first protein.

The agents of the present invention can be, as examples, peptides, small molecules, nucleic acids, vitamin derivatives, as well as carbohydrates. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents of the present invention.

The peptide agents of the invention can be prepared using standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the DNA encoding these peptides may be synthesized using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production using solid phase peptide synthesis is necessitated if non-gene-encoded amino acids are to be included.

Another class of agents are antibodies immunoreactive with one of the proteins under study. Particularly useful are antibodies immunoreactive with the extracellular domain of membrane proteins under study. As described above, antibodies are obtained by immunization of suitable mammalian subjects with peptides, containing as antigenic regions, those portions of the protein intended to be targeted by the antibodies. Critical regions include the contact sites between the two proteins as well as extracellular regions of membrane proteins.

As used herein, the term “agonist” includes those agents, compounds, compositions, etc. which when administered can up-regulate (increase, promote or otherwise elevate the level of) a particular protein.

As used herein, an “allelic variant” refers to a proteins having different amino acid sequences than those sequences listed herein or incorporated by reference. For example, the allelic variants of p27, the target protein interaction domain of p27, SKP1, SKP2 or

SKP2-like proteins, or CUL-1, though possessing a slightly different amino acid sequence, such as a conservative amino acid substitution, than those disclosed herein or incorporated by reference, will still have the requisite biological activity of the native protein. As used herein, a “conservative amino acid substitution” refers to alterations in the amino acid sequence of a protein which do not adversely effect their native abilities. Allelic variants, conservative substitution variants and related proteins and protein fragments utilized herein preferably will have an amino acid sequence having at least about 75% amino acid sequence identity with the published sequences, more preferably at least about 80%, even more preferably at least about 90%, and most preferably at least about 95%.

Thus, the peptides, variants and related molecules that are the subject of or utilized in this invention include molecules having the sequences disclosed; fragments thereof having a consecutive sequence of at least about 3, 5, 10, 15, 20, 25, 30, 50 or more amino acid residues from the corresponding native proteins and amino acid sequence variants of such proteins, or their fragments as defined above, that have been conservatively substituted by another residues.

As used herein, the term “altering the level” of a particular protein means either increasing or decreasing the amount of that protein. For example, “altering the level of SKP2” means either increasing or decreasing the amount of SKP2.

As used herein, the term “antagonist” includes those agents, compounds, compositions, etc. which when administered cause the down regulation (inhibition, prevention, reduction, etc.) of a particular protein.

As used herein, the term “fusion protein” means a hybrid protein including a synthetic or heterologous amino acid sequence. A fusion protein can be produced, for example, from a hybrid gene containing operatively linking heterologous gene sequences.

As used herein, the terms “isolated DNA, RNA, peptides, polypeptides, or proteins” means DNA, RNA, peptides, polypeptides or proteins that are isolated or purified relative to other DNA, RNA, peptides, polypeptides or proteins in the source material. For example, “isolated DNA” that encodes SKP2 (which would include cDNA) refers to DNA purified relative to DNA which encodes polypeptides other than SKP2.

As used herein, the term “modulating the activity” of a particular protein means affecting the covalent or noncovalent binding of that protein with another protein. For

example, when referring to “modulating the activity” of SKP2 this means affecting the binding of SKP2 with another protein, such as p27 or a peptide which includes the SKP2 interaction domain of p27.

5 As used herein, the term “pharmaceutically acceptable” refers to molecular entities and compositions such as fillers and excipients that are physiologically tolerated and do not typically produce an allergic or toxic reaction, such as gastric upset, dizziness and the like when administered to a subject or a patient; the preferred subjects of the invention are vertebrates, mammals and humans.

10 As used herein, the term “polypeptide” refers to a peptide which on hydrolysis yields more than two amino acids, called tripeptides, tetrapeptides, etc. according to the number of amino acids contained in the polypeptide. The term “polypeptide” is used synonymously with the term “protein” and “peptide” throughout the specification.

As used herein, “SCF” refers to a triple protein ligase consisting of SKP, Cullin and F-Box.

15 As used herein, “SCM” refers to a SKP2 C-terminal motif.

As used herein, “SKP” refers to a S-phase kinase associated protein. Specific examples of SKP proteins include, but are not limited to, SKP1, SKP2 and SKP2-like proteins.

20 As used herein, “SKP2-like protein” refers to a protein which can replace SKP2 to form a complex with SKP1 and CUL-1 or their yeast homologs. SKP2-like proteins are proteins that contain a SKP1 interacting domain that is homologous to the SKP1 interacting domain of the SKP2 sequence. Specific examples of SKP2-like proteins include, but are not limited to, ZF1 (SEQ ID NO: 27), ZF3 (SEQ ID NO: 29), ZF4 (SEQ ID NO: 31), ZF5 (SEQ ID NO: 33), ZF6 (SEQ ID NO: 35), ZF7 (SEQ ID NO: 37), ZF8 (SEQ ID NO: 39), ZF9 (SEQ ID NO: 41), ZF11 ( $\beta$ -TRCP) (SEQ ID NO: 43), ZF13 (SEQ ID NO: 45), ZF16 (SEQ ID NO: 47), ZF18 (SEQ ID NO: 49), ZF19 (SEQ ID NO: 51), ZF20 (SEQ ID NO: 53), ZF23 (SEQ ID NO: 55), ZF24 (SEQ ID NO: 57), ZF25 (SEQ ID NO: 59) and ZF26 (SEQ ID NO: 61).

25 As used herein the term “SKP1 interacting domain” refers to the region on the SKP2 protein that interacts with the SKP1 protein. This region is also called the F-box for SKP1 binding.

As used herein the term “SKP2 interacting domain” refers to the region on a protein other than SKP2 that interacts with the SKP2 protein.

As used herein, the term “target protein” refers to an autologous or heterologous protein other than SKP2 which is targeted for interacting with a SKP2 or a SKP2-like protein.

As used herein, the term “target protein interaction domain” refers to a sequence which when fused to SKP2 or a SKP2-like protein interacts with a target protein.

As used herein, the term “ubiquitin” refers to a polypeptide found in all eukaryotic cells that participates in a variety of cellular functions including protein degradation.

As used herein, the terms “ubiquitinating” and “ubiquitination” refer to processes whereby ubiquitin is attached to a protein.

#### B. SKP2-mediated Degradation of Target Proteins

Applicants have identified SKP2 as an F-box protein that mediates ubiquitin-dependent degradation of p27 (SEQ ID NO: 65) and cyclin E (SEQ ID NO: 63). SKP2 (SEQ ID NO: 67) is an F-box protein that is expressed in late G1, S, and G2 phases, playing a role in S phase of the cell cycle (Zhang *et al.*, (1995) Cell 82, 915-925). SCF<sup>SKP2</sup> binds and targets the CDK inhibitor p27 for ubiquitin-dependent degradation. In addition, SKP2 also interacts with cyclin E and plays a role in the ubiquitin-dependent degradation of cyclin E. The present invention therefore includes methods for SKP2-mediated degradation of autologous and heterologous proteins. This SKP2-mediated cyclin E ubiquitination and degradation is mostly dependent on the presence of Thr380 in cyclin E, although weak cyclin E ubiquitination in the absence of Thr380 was also promoted by SKP2 *in vivo*.

Although cyclin E ubiquitination is independent of p27, in the presence of co-expressed CDK inhibitor p27, cyclin E degradation was inhibited even in the presence of SKP2 (Figure 8C). This observation indicates that p27 might inhibit cyclin E autophosphorylation on Thr380, leading to resistance to SKP2-mediated ubiquitin-dependent degradation of cyclin E. The effect of p27 is not to be due to a competition between p27 and cyclin E for SKP2 binding, since a non-phosphorylated mutant form of p27 in which the critical Thr187 was converted into glycine (T187G) cannot bind to



SKP2. This data is consistent with the previous report that p27 inhibits the Thr380-dependent cyclin E degradation (Clurman *et al.*, (1996) Genes Devel. 10, 1979-1990) and indicates that SKP2-mediated cyclin E ubiquitination is p27-independent.

Applicants have also identified that SKP2 performs a dual function during the G1/S transition. It is required for the ubiquitin-dependent degradation of p27 in late G1. The degradation of p27 by SCF<sup>SKP2</sup> activates cyclin E/CDK2 and promotes entry into the S-phase (Sutterluty *et al.*, (1999) Nat. Cell. Biol. 1, 207-14; Coats *et al.*, (1996) Science 272, 877-880). Once cells are in the S phase, cyclin E is degraded which may be required for terminating the S-phase initiation events, allowing the cells to progress from the S phase into the G2 phase (Clurman *et al.*, (1996) Genes Dev. 10, 1979-1990; Won *et al.*, (1996) EMBO J. 15, 4182-4193). Applicants have identified that SKP2 is also involved in the ubiquitin-dependent degradation of cyclin E and therefore the invention encompasses modulation of SKP2 activity and expression as a means of regulating cell cycle progression.

Applicants have determined that a number of phosphorylation dependent and ubiquitin-dependent degradation events occur during the G1/S transition, which are temporally regulated. The expression of SKP2 in the late G1 and S phases leads to assembly of the SCF<sup>SKP2</sup> complex. Previous reports suggest that the phosphorylation status of p27 and cyclin E could be temporally separated. p27 phosphorylation on the critical Thr187 has been shown to occur in the late G1 phase and p27 ubiquitination has been reported to require its binding to the cyclin E/CDK2 complex (Montagnoli *et al.*, (1999) Genes Dev. 13, 1181-1189). The phosphorylation of Thr187 in p27 triggers the binding of SKP2, leading to the subsequent ubiquitin-dependent degradation of p27.

It has been shown that binding of p27 to cyclin E/CDK complexes inhibits the activity of cyclin E/CDK2 and cyclin E degradation (Clurman *et al.*, (1996) Genes Dev. 10, 1979-1990). The binding of p27 therefore prevents phosphorylation on Thr380 in cyclin E or there is a competition between p27 and cyclin E for the binding of SKP2. Applicants have also demonstrated that p27 binding can also cause a conformational change in cyclin E so that Thr380 in cyclin E is not exposed for phosphorylation or SKP2 binding. Applicants have determined that SKP2 binds to the p27 phosphopeptide with higher affinity than that of cyclin E peptide. Thus the affinities between SKP2 and p27 or

cyclin E may also affect the ubiquitination rate of p27 and cyclin E by SKP2. Once p27 is degraded, the cyclin E/CDK2 kinase activity is activated, leading to the S-phase entry. Activation of cyclin E also leads to its autophosphorylation in Thr380 (Clurman *et al.*, (1996) *Genes Dev.* 10, 1979-1990; Won & Reed, (1996) *EMBO J.* 15, 4182-4193). The phosphorylation of Thr380 promotes the SKP2 binding which in turn results in the ubiquitin-dependent degradation of cyclin E. The invention therefore encompasses peptides capable of blocking the interaction of SKP2 and SKP2-like proteins with autologous and heterologous target proteins.

#### 10 C. Alteration of Substrate-Specificity of Various F-box Proteins

F-box proteins are the substrate-targeting component of the SCF complex (SKP1, CUL-1, F-box proteins) (Zhang *et al.*, (1995) *Cell* 82, 915-925; Bai *et al.*, (1996) *Cell* 86, 263-274; Feldman *et al.*, (1997) *Cell* 91, 221-230; Skowyra *et al.*, (1997) *Cell* 91, 209-219). The F-box is a 40-50 amino-acid motif that is commonly present in the otherwise diverse proteins (Zhang *et al.*, (1995) *Cell* 82, 915-925; Bai *et al.*, (1996) *Cell* 86, 263-274). This motif mediates the interaction between an F-box protein and SKP1 (SEQ ID NO: 69). Applicants have identified about 30 F-box proteins which share no apparent homology except in the F-box motif (Figure 12). In mammals, two F-box proteins, SKP2 and  $\beta$ -TRCP, have been well characterized.

Applicants have also determined that SKP2 binds to p27, a CDK inhibitor, through the phosphorylated threonine187 and this interaction targets p27 for ubiquitin-dependent degradation. Applicants further determined that SKP2 interacts with and ubiquitinates cyclin E when the threonine380 of cyclin E is phosphorylated. Likewise it has been shown that  $\beta$ -TRCP (ZF11) binds to two critical serine residues in both  $\beta$ -catenin (serines 33 and 37) and I $\kappa$ B- $\alpha$  (serines 32 and 36) when they are phosphorylated (Maniatis, (1999) *Genes Dev.* 13, 505-510; Winston *et al.*, (1999) *Genes Dev.* 13, 270-283; Spencer *et al.*, (1999) *Genes Dev.* 13, 284-94; Yaron *et al.*, (1998) *Nature* 396, 590-594). This interaction leads to the ubiquitination and degradation of  $\beta$ -catenin or I $\kappa$ B- $\alpha$ . The difference in the substrate binding and thus the substrate specificity by these two F-box proteins is that SKP2 contains a substrate interaction domain of leucine-rich repeats (LRR) at its carboxy-terminal region (residues 220-400) (Zhang *et al.*, (1995) *Cell* 82, 915-25)

while  $\beta$ -TRCP (ZF11) instead has a completely different substrate-interaction domain consisting of WD40 repeats (WD) in the similar position (residues 212-569) (Winston *et al.*, (1999) *Genes Dev* 13, 270-283).

The substrate-specificity of these protein-protein interaction domains has been established through the analysis of yeast F-box proteins such as CDC4, a WD-repeat containing F-box protein, and GRR1, an F-box protein that has LRR at its carboxy terminus (Skowrya *et al.*, (1997) *Cell* 91, 209-219). Although the F-box proteins containing the LRR and WD repeats preferentially bind to substrates only when the substrates are phosphorylated, the existence of many F-box proteins that contain diverse protein-protein interaction domains indicates that many interact with target proteins directly without phosphorylation of the targets Winston *et al.*, (1999) *Curr. Biol.* 9, 1180-1182; Cenciarelli *et al.*, (1999) *Curr. Biol.* 9, 1177-1179).

The fact that various F-box proteins contain completely different substrate-interaction domains indicates that these domains are specifically used to contact substrates. Once the substrates are in association with the F-box proteins, the presence of the F-box region in the F-box proteins promotes the binding of SKP1 and CUL-1 (SEQ ID NO: 71), as well as additional SCF components such as the recently identified Rbx1/Roc1&2 (Ohta *et al.*, (1999) *Mol. Cell.* 3, 535-541; Skowrya *et al.*, (1999) *Science* 284, 662-665; Kamura *et al.*, (1999) *Science* 284, 657-661), to form the SCF complexes. The assembly of the complete SCF ubiquitin E3 ligase complexes promotes the ubiquitin-transfer reaction to the SCF-interacting substrates by the ubiquitin conjugating E2 enzyme, CDC34, and the ubiquitin activating enzyme E1 (Koepp *et al.*, (1999) *Cell* 97, 431-434). The polyubiquitinated substrate proteins are subsequently degraded by the 26S proteasome.

In the case of SKP2 and  $\beta$ -TRCP, the effect of mutation in the F-box region has been examined. Expression of mutant forms of SKP2 or  $\beta$ -TRCP that contain a deletion in the F-box but retain the complete substrate-interaction domain of LRR or WD repeats causes the protection of their respective substrates, p27, cyclin E or  $\beta$ -catenin and I $\kappa$ B (Carrano *et al.*, (1999) *Nat. Cell. Biol.* 1, 193-199; Winston *et al.*, (1999) *Genes Dev.* 13, 270-283; Spencer *et al.*, (1999) *Genes Dev.* 13, 284-294) (Figure 9). This is because these SKP2 or  $\beta$ -TRCP mutants are fully capable of binding to the substrates while defective in

recruiting the SKP1/CUL-1 into the complex, producing a dominant negative effect for the stability of the target proteins *in vivo*.

The concept of altering the substrate specificity of the various F-box proteins can thus be extended to fuse a protein interaction domain or a ligand binding site, in the form of either a protein, a peptide, or a chemical, with the F-box motif of either SKP2,  $\beta$ -TRCP (ZF11) or other F-box proteins (ZF series). In this design, this hybrid protein or molecule can be used to bind its normal protein partner and targets the protein partner for ubiquitin-dependent degradation. For example, if the F-box protein is fused with Max or Mad, proteins that bind to Myc oncoprotein (Blackwood & Eisenman, (1991) Science 251, 1211-1217; Blackwood *et al.*, (1991) Cold Spring Harb. Symp. Quant. Biol. 56, 109-117), the F-box/Max or Mad fusion protein will bind to and target Myc for ubiquitination and degradation.

Thus in one aspect of the invention, the protein levels of Myc in a cell can be modulated by such an F-box/Max or Mad hybrid construct. Another example is fusion of the amino-terminus of MDM2 (residues 1-158), a region that is known to bind the tumor suppressor protein p53 (Chen *et al.*, (1993) Mol. Cell. Biol. 13, 4107-4114), with the F-box region derived from SKP2,  $\beta$ -TRCP and other F-box proteins. A hybrid F-box/MDM2 protein could be generated that would target p53 for ubiquitination. Such a pairwise selection can be extended to the cyclin-CDK (Hunter & Pines, (1994) Cell 79, 573-582), Bcl-2-Bax/Bad (Yang *et al.*, (1995) Cell 80, 285-291; Chao & Korsmeyer, (1998) Annu. Rev. Immunol. 16, 395-419), and many others for the selective degradation of the desired targets.

The concept of modulating protein levels by the alteration of SCF substrate-targeting specificity can be further extended to include fusing the protein-interaction domains with a peptide or a chemical that interact with SKP1 or CUL1 or the SCF complex. A fusion protein is an expression product resulting from the fusion of two genes. Such a protein may be produced, *e.g.*, in recombinant DNA expression studies or, naturally, in certain viral oncogenes in which the oncogene is fused to *gag*.

The production of a fusion protein sometimes results from the need to place a cloned eukaryotic gene under the control of a bacterial promoter for expression in a bacterial system. Sequences of the bacterial system are then frequently expressed linked

to the eukaryotic protein. Fusion proteins are used for the analysis of structure, purification, function, and expression of heterologous gene products.

A fused protein is a hybrid protein molecule which can be produced when a nucleic acid of interest is inserted by recombinant DNA techniques into a recipient plasmid and displaces the stop codon for a plasmid gene. The fused protein begins at the amino end with a portion of the plasmid protein sequence and ends with the protein of interest.

The production of fusion proteins is well known to one skilled in the art (see U.S. Patent Numbers 5,908,756; 5,907,085; 5,906,819; 5,905,146; 5,895,813; 5,891,643; 5,891,628; 5,891,432; 5,889,169; 5,889,150; 5,888,981; 5,888,773; 5,886,150; 5,886,149; 5,885,833; 5,885,803; 5,885,779; 5,885,580; 5,883,124; 5,882,941; 5,882,894; 5,882,864; 5,879,917; 5,879,893; 5,876,972; 5,874,304; and 5,874,290). For a general review of the construction, properties, applications and problems associated with specific types of fusion molecules used in clinical and research medicine, see Chamow *et al.*, (1999) Antibody Fusion Proteins, John Wiley.

#### D. Modulation of SKP2 Expression and Activity

The identification of SKP2 and SKP2-like proteins has led to the discovery of compounds that are capable of down-regulating expression of these proteins. Molecules that down-regulate SKP2 and SKP2-like proteins are therefore part of the invention.

Down-regulation is defined here as a decrease in activation, function or synthesis of SKP2 and SKP2-like proteins, its ligands or activators. It is further defined to include an increase in the degradation of the SKP2 gene, its protein product, ligands or activators.

Down-regulation is therefore achieved in a number of ways. For example, administration of molecules that can destabilize the binding of SKP2 and SKP2-like proteins with its ligands. Such molecules encompass polypeptide products, including those encoded by the DNA sequences of the SKP2 gene or DNA sequences containing various mutations.

These mutations may be point mutations, insertions, deletions or spliced variants of the SKP2 gene. This invention also includes truncated polypeptides encoded by the DNA molecules described above. These polypeptides being capable of interfering with interaction of SKP2 and SKP2-like proteins with other proteins.

A further embodiment of this invention includes the down-regulation of SKP2 function by altering expression of the SKP2 gene, the use of antisense gene therapy being an example. Down-regulation of SKP2 or SKP2-like protein expression is accomplished by administering an effective amount of antisense oligonucleotides. These antisense molecules can be fashioned from the DNA sequence of the SKP2 gene or sequences containing various mutations, deletions, insertions or spliced variants. Another embodiment of this invention relates to the use of isolated RNA or DNA sequences derived from the SKP2 gene. These sequences containing various mutations such as point mutations, insertions, deletions or spliced variant mutations of SKP2 gene and can be useful in gene therapy.

Molecules that increase the degradation of the SKP2 or SKP2-like proteins may also be used to down-regulate its functions and are within the scope of the invention. Phosphorylation of SKP2 or SKP2-like proteins may alter protein stability, therefore kinase inhibitors may be used to down-regulate its function. Down-regulation of SKP2 or SKP2-like proteins may also be accomplished by the use of polyclonal or monoclonal antibodies or fragments thereof directed against the SKP2 or SKP2-like proteins. Such molecules are within the claimed invention. This invention further includes small molecules with the three-dimensional structure necessary to bind with sufficient affinity to block SKP2 or SKP2-like protein interactions with p27 or cyclin E. SKP2 or SKP2-like protein blockade resulting in decreased degradation of p27 or cyclin E and other processes of transformed cells where it is expressed make these small molecules useful as therapeutic agents in treating tumors.

The agents discussed above represent various effective therapeutic compounds in treating tumors. Applicants have thus provided antagonists and methods of identifying antagonists that are capable of down-regulating SKP2 or SKP2-like proteins.

A further embodiment of the invention relates to antisense or gene therapy. It is now known in the art that altered DNA molecules can be tailored to provide a specific selected effect, when provided as antisense or gene therapy. The native DNA segment coding for SKP2 has, as do all other mammalian DNA strands, two strands; a sense strand and an antisense strand held together by hydrogen bonds. The mRNA coding for SKP2 has a nucleotide sequence identical to the sense strand, with the expected substitution of

thymidine by uridine. Thus, based upon the knowledge of the SKP2 sequence, synthetic oligonucleotides can be synthesized. These oligonucleotides can bind to the DNA and RNA coding for SKP2. The active fragments of the invention, which are complementary to mRNA and the coding strand of DNA, are usually at least about 15 nucleotides, more  
5 usually at least 20 nucleotides, preferably 30 nucleotides and more preferably may be 50 nucleotides or more. There is no upper limit, other than a practical limit, on the maximal size of such a nucleic acid molecule in that the nucleic acid molecule can include a portion of a gene, an entire gene, or multiple genes, or portions thereof. The binding strength between the sense and antisense strands is dependent upon the total hydrogen bonds.  
10 Therefore, based upon the total number of bases in the mRNA, the optimal length of the oligonucleotide sequence may be easily calculated by the skilled artisan. The sequence may be complementary to any portion of the sequence of the mRNA. For example, it may be proximal to the 5'-terminus or capping site or downstream from the capping site, between the capping site and the initiation codon and may cover all or only a portion of  
15 the non-coding region or the coding region. The particular site(s) to which the antisense sequence binds will vary depending upon the degree of inhibition desired, the uniqueness of the sequence, the stability of the antisense sequence, etc.

In the practice of the invention, expression of SKP2 or SKP2-like proteins are down-regulated by administering an effective amount of synthetic antisense  
20 oligonucleotide sequences described above. The oligonucleotide compounds of the invention bind to the mRNA coding for human SKP2 thereby inhibiting expression (translation) of these proteins. The isolated DNA sequences containing various mutations such as point mutations, insertions, deletions or spliced mutations of SKP2 are useful in gene therapy as well.

25 Antisense oligonucleotides can also be used as tools *in vitro* to determine the biological function of genes and proteins. Oligonucleotide phosphorothioates (PS-oligos) have also shown great therapeutic potential as antisense-mediated inhibitors of gene expression. Various methods have been developed for the synthesis of antisense oligonucleotides. See Agrawal *et al.*, (1993) *Methods of Molecular Biology: Protocols for*  
30 *Oligonucleotides and Analogs*, Humana Press; Eckstein *et al.*, (1991) *Oligonucleotides and Analogues: A Practical Approach*, Oxford University Press).

### E. Diagnostic Assays

In another diagnostic embodiment, susceptibility to certain tumors associated with elevated levels of SKP2 or SKP2-like proteins in a human subject can be measured by the steps of: (a) measuring the level of SKP2 or SKP2-like proteins in a biological sample from said human subject; and (b) comparing the level of SKP2 or SKP2-like proteins present in normal subjects, wherein an increase in the level of SKP2 or SKP2-like proteins as compared to normal levels indicates a predisposition to certain tumors.

In another diagnostic embodiment, a therapeutic treatment of certain tumors associated with elevated levels of SKP2 or SKP2-like proteins in a human subject may be monitored by measuring the levels of SKP2 or SKP2-like proteins in a series of biologic samples obtained at different time points from said subject undergoing therapeutic treatment wherein a significant decrease in said levels of SKP2 or SKP2-like proteins indicates a successful therapeutic treatment.

Diagnostic probes useful in such assays of the invention include antibodies to SKP2 or SKP2-like proteins. The antibodies to SKP2 or SKP2-like proteins may be either monoclonal or polyclonal, produced using standard techniques well known in the art (See Harlow & Lane, (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press). They can be used to detect SKP2 or SKP2-like proteins by binding to the protein and subsequent detection of the antibody-protein complex by ELISA, Western blot or the like. The SKP2 or SKP2-like proteins used to elicit these antibodies can be any of the SKP2 or SKP2-like proteins variants discussed above. Antibodies are also produced from peptide sequences of SKP2 or SKP2-like proteins using standard techniques in the art (See Protocols in Immunology, John Wiley & Sons, 1994). Fragments of the monoclonals or the polyclonal antisera which contain the immunologically significant portion can also be prepared. Use of immunologically reactive fragments, such as the Fab, Fab', of F(ab')<sub>2</sub> fragments is often preferable, especially in a therapeutic context, as these fragments are generally less immunogenic than the whole immunoglobulin.

Assays to detect or measure SKP2 or SKP2-like proteins polypeptide in a biological sample with an antibody probe may be based on any available format. For instance, in immunoassays where SKP2 or SKP2-like proteins are the analyte, the test



sample, typically a biological sample, is incubated with anti-SKP2 antibodies under conditions that allow the formation of antigen-antibody complexes. Various formats can be employed, such as "sandwich" assay where antibody bound to a solid support is incubated with the test sample; washed, incubated with a second, labeled antibody to the  
5 analyte; and the support is washed again. Analyte is detected by determining if the second antibody is bound to the support. In a competitive format, which can be either heterogeneous or homogeneous, a test sample is usually incubated with an antibody and a labeled competing antigen, either sequentially or simultaneously. These and other formats are well known in the art.

10

#### F. Methods to Identify Binding Partners

Another embodiment of the present invention provides methods for use in isolating and identifying binding partners of proteins of the invention. In detail, a protein of the invention is mixed with a potential binding partner or an extract or fraction of a cell under  
15 conditions that allow the association of potential binding partners with the protein of the invention. After mixing, peptides, polypeptides, proteins or other molecules that have become associated with a protein of the invention are separated from the mixture. The binding partner bound to the protein of the invention can then be removed and further analyzed. To identify and isolate a binding partner, the entire protein, for instance the  
20 entire SKP2 or SKP2-like protein can be used. Alternatively, a fragment of the protein can be used, such as the SKP-1 interacting domain.

As used herein, a cellular extract refers to a preparation or fraction which is made from a lysed or disrupted cell. The preferred source of cellular extracts will be cells derived from human tissue, for instance, malignant tissue. Alternatively, cellular extracts  
25 may be prepared from any source of malignant tissue or available cell lines.

A variety of methods can be used to obtain an extract of a cell. Cells can be disrupted using either physical or chemical disruption methods. Examples of physical disruption methods include, but are not limited to, sonication and mechanical shearing. Examples of chemical lysis methods include, but are not limited to, detergent lysis and  
30 enzyme lysis. A skilled artisan can readily adapt methods for preparing cellular extracts in order to obtain extracts for use in the present methods.

Once an extract of a cell is prepared, the extract is mixed with the protein of the invention under conditions in which association of the protein with the binding partner can occur. A variety of conditions can be used, the most preferred being conditions that closely resemble conditions found in the cytoplasm of a human cell. Features such as osmolarity, pH, temperature, and the concentration of cellular extract used, can be varied to optimize the association of the protein with the binding partner.

After mixing under appropriate conditions, the bound complex is separated from the mixture. A variety of techniques can be utilized to separate the mixture. For example, antibodies specific to a protein of the invention can be used to immunoprecipitate the binding partner complex. Alternatively, standard chemical separation techniques such as chromatography and density-sediment centrifugation can be used.

After removal of non-associated cellular constituents found in the extract, the binding partner can be dissociated from the complex using conventional methods. For example, dissociation can be accomplished by altering the salt concentration or pH of the mixture.

To aid in separating associated binding partner pairs from the mixed extract, the protein of the invention can be immobilized on a solid support. For example, the protein can be attached to a nitrocellulose matrix or acrylic beads. Attachment of the protein to a solid support aids in separating peptide-binding partner pairs from other constituents found in the extract. The identified binding partners can be either a single protein or a complex made up of two or more proteins. Alternatively, binding partners may be identified using the Alkaline Phosphatase fusion assay according to the procedures of Flanagan & Vanderhaeghen, (1998) *Annu. Rev. Neurosci.* 21, 309-345 or Takahashi *et al.*, (1999) *Cell* 99, 59-69; the Far-Western assay according to the procedures of Takayama *et al.*, (1997) *Methods Mol. Biol.* 69, 171-184 or Sauder *et al.*, *J. Gen. Virol.* (1996) 77, 991-996 or identified through the use of epitope tagged proteins or GST fusion proteins.

Alternatively, the nucleic acid molecules of the invention can be used in a yeast two-hybrid system. The yeast two-hybrid system has been used to identify other protein partner pairs and can readily be adapted to employ the nucleic acid molecules herein described (see Stratagene Hybrizap® two-hybrid system).

### G. Methods to Identify Agents that Modulate Expression

Another embodiment of the present invention provides methods for identifying agents that modulate the expression of a nucleic acid encoding the SKP2 protein, or of a nucleic acid encoding the SKP2 or SKP2-like protein such as a protein. Such assays may  
5 utilize any available means of monitoring for changes in the expression level of the nucleic acids of the invention. As used herein, an agent is said to modulate the expression of a nucleic acid, for instance a nucleic acid encoding the protein having the sequence of SKP2, SKP2-like proteins, SKP1, CUL-1, or any F-box containing protein such as a ZF protein, if it is capable of up- or down-regulating expression of the nucleic acid in a cell.

10 In one assay format, cell lines that contain reporter gene fusions between the open reading frame of SKP2 or a SKP2-like protein and any assayable fusion partner may be prepared. Numerous assayable fusion partners are known and readily available including the firefly luciferase gene and the gene encoding chloramphenicol acetyltransferase (Alam *et al.*, (1990) *Anal. Biochem.* 188, 245-254). Cell lines containing the reporter gene  
15 fusions are then exposed to the agent to be tested under appropriate conditions and time. Differential expression of the reporter gene between samples exposed to the agent and control samples identifies agents which modulate the expression of a nucleic acid encoding an SKP2, SKP2-like or ZF protein.

Additional assay formats may be used to monitor the ability of the agent to  
20 modulate the expression of a nucleic acid encoding a SKP-2 or SKP2-like protein. For instance, mRNA expression may be monitored directly by hybridization to the nucleic acids of the invention. Cell lines are exposed to the agent to be tested under appropriate conditions and time and total RNA or mRNA is isolated by standard procedures such those disclosed in Sambrook *et al.*, (1989) *Molecular Cloning - A Laboratory Manual*, Cold  
25 Spring Harbor Laboratory Press.

Probes to detect differences in RNA expression levels between cells exposed to the agent and control cells may be prepared from the nucleic acids of the invention. It is preferable, but not necessary, to design probes which hybridize only with target nucleic acids under conditions of high stringency. Only highly complementary nucleic acid  
30 hybrids form under conditions of high stringency. Accordingly, the stringency of the assay conditions determines the amount of complementarity which should exist between

two nucleic acid strands in order to form a hybrid. Stringency should be chosen to maximize the difference in stability between the probe:target hybrid and potential probe:non-target hybrids.

Probes may be designed from the nucleic acids of the invention through methods known in the art. For instance, the G+C content of the probe and the probe length can affect probe binding to its target sequence. Methods to optimize probe specificity are commonly available in Sambrook *et al.*, (1989) Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratory Press or Ausubel *et al.*, (1995) Current Protocols in Molecular Biology, Greene Publishing.

Hybridization conditions are modified using known methods, such as those described by Sambrook *et al.*, (1989) and Ausubel *et al.*, (1995) as required for each probe. Hybridization of total cellular RNA or RNA enriched for polyA+ RNA can be accomplished in any available format. For instance, total cellular RNA or RNA enriched for polyA+ RNA can be affixed to a solid support and the solid support exposed to at least one probe comprising at least one, or part of one of the sequences of the invention under conditions in which the probe will specifically hybridize. Alternatively, nucleic acid fragments comprising at least one, or part of one of the sequences of the invention can be affixed to a solid support, such as a silicon based wafer or a porous glass wafer. The wafer can then be exposed to total cellular RNA or polyA+ RNA from a sample under conditions in which the affixed sequences will specifically hybridize. Such wafers and hybridization methods are widely available, for example, those disclosed by Beattie, (WO9511755). By examining for the ability of a given probe to specifically hybridize to a RNA sample from an untreated cell population and from a cell population exposed to the agent, agents which up or down regulate the expression of a nucleic acid encoding the SKP2 protein are identified.

Hybridization for qualitative and quantitative analysis of mRNA may also be carried out by using a RNase Protection Assay (*i.e.*, RPA, see Ma *et al.*, Methods (1996) 10, 273-238). Briefly, an expression vehicle comprising cDNA encoding the gene product and a phage specific DNA dependent RNA polymerase promoter (*e.g.*, T7, T3 or SP6 RNA polymerase) is linearized at the 3' end of the cDNA molecule, downstream from the phage promoter, wherein such a linearized molecule is subsequently used as a template for

synthesis of a labeled antisense transcript of the cDNA by *in vitro* transcription. The labeled transcript is then hybridized to a mixture of isolated RNA (*i.e.*, total or fractionated mRNA) by incubation at 45°C overnight in a buffer comprising 80% formamide, 40 mM Pipes, pH 6.4, 0.4 M NaCl and 1 mM EDTA. The resulting hybrids are then digested in a buffer comprising 40 µg/ml ribonuclease A and 2 µg/ml ribonuclease. After deactivation and extraction of extraneous proteins, the samples are loaded onto urea-polyacrylamide gels for analysis.

In another assay format, agents which effect the expression of the instant gene products, cells or cell lines would first be identified which express said gene products physiologically. Cells and cell lines so identified would be expected to comprise the necessary cellular machinery such that the fidelity of modulation of the transcriptional apparatus is maintained with regard to exogenous contact of agent with appropriate surface transduction mechanisms and the cytosolic cascades. Further, such cells or cell lines would be transduced or transfected with an expression vehicle (*e.g.*, a plasmid or viral vector) construct comprising an operable non-translated 5'-promoter containing end of the structural gene encoding the instant gene products fused to one or more antigenic fragments, which are peculiar to the instant gene products, wherein said fragments are under the transcriptional control of said promoter and are expressed as polypeptides whose molecular weight can be distinguished from the naturally occurring polypeptides or may further comprise an immunologically distinct tag. Such a process is well known in the art (see, Sambrook *et al.*, (1989) Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratory Press).

Cells or cell lines transduced or transfected as outlined above would then be contacted with agents under appropriate conditions; for example, the agent comprises a pharmaceutically acceptable excipient and is contacted with cells in an aqueous physiological buffer such as phosphate buffered saline (PBS) at physiological pH, Eagles balanced salt solution (BSS) at physiological pH, PBS or BSS comprising serum or conditioned media comprising PBS or BSS and serum incubated at 37°C. Said conditions may be modulated as deemed necessary by one of skill in the art. Subsequent to contacting the cells with the agent, said cells will be disrupted and the polypeptides of the disruptate are fractionated such that a polypeptide fraction is pooled and contacted with an antibody

to be further processed by immunological assay (e.g., ELISA, immunoprecipitation or Western blot). The pool of proteins isolated from the "agent contacted" sample will be compared with a control sample where only the excipient is contacted with the cells and an increase or decrease in the immunologically generated signal from the "agent contacted" sample compared to the control will be used to distinguish the effectiveness of the agent.

#### H. Methods to Identify Agents that Modulate Activity

Another embodiment of the present invention provides methods for identifying agents that modulate at least one activity of a protein of the invention such as SKP2 or SKP2-like proteins. Such methods or assays may utilize any means of monitoring or detecting the desired activity.

The present invention includes methods of screening for compounds which deactivate, or act as antagonists of SKP2 or SKP2-like protein expression. Such compounds may be useful in the modulation of pathological conditions associated with alterations in SKP2, SKP2-like or p27 protein levels.

In one format, the relative amounts of a SKP2 protein between a cell population that has been exposed to the agent to be tested compared to an un-exposed control cell population may be assayed. In this format, probes such as specific antibodies are used to monitor the differential expression of the protein in the different cell populations. Cell lines or populations are exposed to the agent to be tested under appropriate conditions and time. Cellular lysates may be prepared from the exposed cell line or population and a control, unexposed cell line or population. The cellular lysates are then analyzed with the probe.

Antibody probes are prepared by immunizing suitable mammalian hosts in appropriate immunization protocols using the SKP2 or SKP2-like proteins if they are of sufficient length, or if desired, or if required to enhance immunogenicity, conjugated to suitable carriers. Methods for preparing immunogenic conjugates with carriers such as BSA, KLH, or other carrier proteins are well known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents may be effective; in other instances linking reagents such as those supplied by Pierce Chemical Co. may be desirable to provide accessibility to the hapten. The hapten peptides can be extended at either the

amino or carboxy terminus with a cysteine residue or interspersed with cysteine residues, for example, to facilitate linking to a carrier. Administration of the immunogens is conducted generally by injection over a suitable time period and with use of suitable adjuvants, as is generally understood in the art. During the immunization schedule, titers  
5 of antibodies are taken to determine adequacy of antibody formation.

While the polyclonal antisera produced in this way may be satisfactory for some applications, for pharmaceutical compositions, use of monoclonal preparations is preferred. Immortalized cell lines which secrete the desired monoclonal antibodies may be prepared using the standard method of Kohler & Milstein, (1992) *Biotechnology* 24, 524-  
10 526 or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known. The immortalized cell lines secreting the desired antibodies are screened by immunoassay in which the antigen is the peptide hapten, polypeptide or protein. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells can be cultured either *in vitro* or by production in ascites fluid.

15 The desired monoclonal antibodies may be recovered from the culture supernatant or from the ascites supernatant. Fragments of the monoclonals or the polyclonal antisera which contain the immunologically significant portion can be used as antagonists, as well as the intact antibodies. Use of immunologically reactive fragments, such as the Fab, Fab' of F(ab')<sub>2</sub> fragments is often preferable, especially in a therapeutic context, as these  
20 fragments are generally less immunogenic than the whole immunoglobulin.

The antibodies or fragments may also be produced, using current technology, by recombinant means. Antibody regions that bind specifically to the desired regions of the protein can also be produced in the context of chimeras with multiple species origin.

25 Antibody regions that bind specifically to the desired regions of the protein can also be produced in the context of chimeras with multiple species origin, for instance, humanized antibodies. The antibody can therefore be a humanized antibody or human a antibody, as described in U. S. Patent No. 5,585,089 or Riechmann *et al.*, (1988) *Nature* 332, 323-327.

30 Agents that are assayed in the above method can be randomly selected or rationally selected or designed. As used herein, an agent is said to be randomly selected when the agent is chosen randomly without considering the specific sequences involved in the

association of the a protein of the invention alone or with its associated substrates, binding partners, etc. An example of randomly selected agents is the use a chemical library or a peptide combinatorial library, or a growth broth of an organism.

As used herein, an agent is said to be rationally selected or designed when the agent is chosen on a non-random basis which takes into account the sequence of the target site or its conformation in connection with the agent's action. Agents can be rationally selected or rationally designed by utilizing the peptide sequences that make up these sites. For example, a rationally selected peptide agent can be a peptide whose amino acid sequence is identical to the SKP1 or SKP2 interaction domain on a autologous or heterologous target protein which interacts with the SKP2 protein or its targets.

The agents of the present invention can be, as examples, peptides, small molecules, vitamin derivatives, as well as carbohydrates. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents of the present invention.

The peptide agents of the invention can be prepared using standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the DNA encoding these peptides may be synthesized using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production using solid phase peptide synthesis is necessitated if non-gene-encoded amino acids are to be included.

Another class of agents of the present invention are antibodies immunoreactive with critical positions of proteins of the invention. For example, antibodies which specifically interact with the SKP1 interacting domain, SKP2 interacting domain or the SKP2 C-terminal motif. Antibody agents are obtained by immunization of suitable mammalian subjects with peptides, containing as antigenic regions, those portions of the protein intended to be targeted by the antibodies.

#### I. High Throughput Assays

The power of high throughput screening is utilized to the search for new compounds which are capable of interacting with the SKP2 or SKP-2 like proteins. For general information on high-throughput screening (see Devlin, (1998) High Throughput



Screening, Marcel Dekker; U.S. Patent No. 5,763,263). High throughput assays utilize one or more different assay techniques.

Immunodiagnosics and Immunoassays. These are a group of techniques used for the measurement of specific biochemical substances, commonly at low concentrations in complex mixtures such as biological fluids, that depend upon the specificity and high affinity shown by suitably prepared and selected antibodies for their complementary antigens. A substance to be measured must, of necessity, be antigenic -either an immunogenic macromolecule or a haptenic small molecule. To each sample a known, limited amount of specific antibody is added and the fraction of the antigen combining with it, often expressed as the bound:free ratio, is estimated, using as indicator a form of the antigen labeled with radioisotope (radioimmunoassay), fluorescent molecule (fluoroimmunoassay), stable free radical (spin immunoassay), enzyme (enzyme immunoassay), or other readily distinguishable label.

Antibodies can be labeled in various ways, including: enzyme-linked immunosorbent assay (ELISA); radioimmuno assay (RIA); fluorescent immunoassay (FIA); chemiluminescent immunoassay (CLIA); and labeling the antibody with colloidal gold particles (immunogold).

Common assay formats include the sandwich assay, competitive or competition assay, latex agglutination assay, homogeneous assay, microtitre plate format and the microparticle-based assay.

Enzyme-linked immunosorbent assay (ELISA). ELISA is an immunochemical technique that avoids the hazards of radiochemicals and the expense of fluorescence detection systems. Instead, the assay uses enzymes as indicators. ELISA is a form of quantitative immunoassay based on the use of antibodies (or antigens) that are linked to an insoluble carrier surface, which is then used to "capture" the relevant antigen (or antibody) in the test solution. The antigen-antibody complex is then detected by measuring the activity of an appropriate enzyme that had previously been covalently attached to the antigen (or antibody).

For information on ELISA techniques, see, for example, Crowther, (1995) ELISA - Theory and Practice (Methods in Molecular Biology), Humana Press; Challacombe & Kemeny, (1998) ELISA and Other Solid Phase Immunoassays - Theoretical and Practical

Aspects, John Wiley; Kemeny, (1991) A Practical Guide to ELISA, Pergamon Press; Ishikawa, (1991) Ultrasensitive and Rapid Enzyme Immunoassay (Laboratory Techniques in Biochemistry and Molecular Biology) Elsevier.

Colorimetric Assays for Enzymes. Colorimetry is any method of quantitative chemical analysis in which the concentration or amount of a compound is determined by comparing the color produced by the reaction of a reagent with both standard and test amounts of the compound, often using a colorimeter. A colorimeter is a device for measuring color intensity or differences in color intensity, either visually or photoelectrically.

Standard colorimetric assays of beta-galactosidase enzymatic activity are well known to those skilled in the art (see, for example, Norton *et al.*, (1985) Mol. Cell. Biol. 5, 281-290). A colorimetric assay can be performed on whole cell lysates using O-nitrophenyl-beta-D-galactopyranoside (ONPG, Sigma) as the substrate in a standard colorimetric beta-galactosidase assay (Sambrook *et al.*, (1989) Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratory Press. Automated colorimetric assays are also available for the detection of beta-galactosidase activity, as described in U.S. Patent No. 5,733,720.

Immunofluorescence Assays. Immunofluorescence or immunofluorescence microscopy is a technique in which an antigen or antibody is made fluorescent by conjugation to a fluorescent dye and then allowed to react with the complementary antibody or antigen in a tissue section or smear. The location of the antigen or antibody can then be determined by observing the fluorescence by microscopy under ultraviolet light.

For general information on immunofluorescent techniques, see, for example, Knapp *et al.*, (1978) Immunofluorescence and Related Staining Techniques, Elsevier; Allan, (1999) Protein Localization by Fluorescent Microscopy - A Practical Approach (The Practical Approach Series) Oxford University Press; Caul, (1993) Immunofluorescence Antigen Detection Techniques in Diagnostic Microbiology, Cambridge University Press. For detailed explanations of immunofluorescent techniques applicable to the present invention, see U.S. Patent Nos. 5,912,176; 5,869,264; 5,866,319; 5,861,259.

### J. Pharmaceutical preparations

The invention also includes pharmaceutical compositions comprising the compounds of the invention together with a pharmaceutically acceptable carrier.

Pharmaceutically acceptable carriers can be sterile liquids, such as water and oils,

5 including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in Remington's  
10 Pharmaceutical Sciences, Mack Publishing Company, 1995. In addition to the pharmacologically active agent, the compositions of the present invention may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically for delivery to the site of action. Suitable formulations for parenteral  
15 administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain  
20 substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers. Liposomes can also be used to encapsulate the agent for delivery into the cell.

The pharmaceutical formulation for systemic administration according to the  
25 invention may be formulated for enteral, parenteral or topical administration. Indeed, all three types of formulations may be used simultaneously to achieve systemic administration of the active ingredient.

Suitable formulations for oral administration include hard or soft gelatin capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and  
30 controlled release forms thereof.

The agents of the present invention can be administered via parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, or buccal routes. Alternatively, or concurrently, administration may be by the oral route or directly to the lungs. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

The compounds used in the method of treatment of this invention may be administered systemically or topically, depending on such considerations as the condition to be treated, need for site-specific treatment, quantity of drug to be administered and similar considerations.

Topical administration may be used. Any common topical formation such as a solution, suspension, gel, ointment or salve and the like may be employed. Preparation of such topical formulations are well described in the art of pharmaceutical formulations as exemplified, for example, by Remington's Pharmaceutical Sciences. For topical application, these compounds could also be administered as a powder or spray, particularly in aerosol form. The active ingredient may be administered in pharmaceutical compositions adapted for systemic administration. As is known, if a drug is to be administered systemically, it may be confected as a powder, pill, tablet or the like or as a syrup or elixir for oral administration. For intravenous, intraperitoneal or intra-lesional administration, the compound will be prepared as a solution or suspension capable of being administered by injection. In certain cases, it may be useful to formulate these compounds in suppository form or as an extended release formulation for deposit under the skin or intramuscular injection. In a preferred embodiment, the compounds of this invention may be administered by inhalation. For inhalation therapy the compound may be in a solution useful for administration by metered dose inhalers or in a form suitable for a dry powder inhaler.

An effective amount is that amount which will modulate the activity or alter the level of a target protein. A given effective amount will vary from condition to condition and in certain instances may vary with the severity of the condition being treated and the patient's susceptibility to treatment. Accordingly, a given effective amount will be best determined at the time and place through routine experimentation. However, it is

anticipated that in the treatment of a tumor in accordance with the present invention, a formulation containing between 0.001 and 5 percent by weight, preferably about 0.01 to 1 percent, will usually constitute a therapeutically effective amount. When administered systemically, an amount between 0.01 and 100 mg per kg body weight per day, but preferably about 0.1 to 10 mg/kg, will effect a therapeutic result in most instances.

In practicing the methods of this invention, the compounds of this invention may be used alone or in combination, or in combination with other therapeutic or diagnostic agents. In certain preferred embodiments, the compounds of this invention may be coadministered along with other compounds typically prescribed for these conditions according to generally accepted medical practice. The compounds of this invention can be utilized *in vivo*, ordinarily in mammals, preferably in humans.

In still another embodiment, the compounds of the invention may be coupled to chemical moieties, including proteins that alter the functions or regulation of target proteins for therapeutic benefit. These proteins may include in combination other inhibitors of cytokines and growth factors that may offer additional therapeutic benefit in the treatment of tumors. In addition, the molecules of the invention may also be conjugated through phosphorylation to biotinylate, thioate, acetylate, iodinate using any of the cross-linking reagents well known in the art.

#### K. Transgenic Animals

The term "animal" as used herein includes all vertebrate animals, except humans. It also includes an individual animal in all stages of development, including embryonic and fetal stages. A "transgenic animal" is an animal containing one or more cells bearing genetic information received, directly or indirectly, by deliberate genetic manipulation at a subcellular level, such as by microinjection or infection with recombinant virus. This introduced DNA molecule may be integrated within a chromosome, or it may be extra-chromosomally replicating DNA. The term "germ cell-line transgenic animal" refers to a transgenic animal in which the genetic information was introduced into a germ line cell, thereby conferring the ability to transfer the information to offspring. If such offspring in fact possess some or all of that information, then they, too, are transgenic animals. Transgenic animals containing mutant, knock-out, modified genes or gene

constructs to over-express or conditionally express a gene corresponding to the cDNA sequence of SEQ ID NO: 66 or related sequences are encompassed in the invention.

The information may be foreign to the species of animal to which the recipient belongs, foreign only to the particular individual recipient, or genetic information already  
5 possessed by the recipient. In the last case, the introduced gene may be differently expressed compared to the native endogenous gene. The genes may be obtained by isolating them from genomic sources, by preparation of cDNA from isolated RNA templates, by directed synthesis, or by some combination thereof.

To be expressed, a gene should be operably linked to a regulatory region.  
10 Regulatory regions, such as promoters, may be used to increase, decrease, regulate or designate to certain tissues or to certain stages of development the expression of a gene. The promoter need not be a naturally occurring promoter. The "transgenic non-human animals" of the invention are produced by introducing "transgenes" into the germline of the non-human animal. The methods enabling the introduction of DNA into cells are  
15 generally available and well-known in the art. Different methods of introducing transgenes could be used. Generally, the zygote is the best target for microinjection. In the mouse, the male pronucleus reaches the size of approximately twenty microns in diameter, which allows reproducible injection of one to two picoliters of DNA solution. The use of zygotes as a target for gene transfer has a major advantage. In most cases, the  
20 injected DNA will be incorporated into the host gene before the first cleavage (Brinster *et al.*, (1985) Proc. Natl. Acad. Sci. USA 82, 4438-4442.). Consequently, nearly all cells of the transgenic non-human animal will carry the incorporated transgene. Generally, this will also result in the efficient transmission of the transgene to offspring of the founder since 50% of the germ cells will harbor the transgene. Microinjection of zygotes is a  
25 preferred method for incorporating transgenes in practicing the invention.

Retroviral infection can also be used to introduce a transgene into a non-human animal. The developing non-human embryo can be cultured *in vitro* to the blastocyst stage. During this time, blastomeres may be targets for retroviral infection. Efficient infection of the blastomeres is obtained by enzymatic treatment to remove the zona  
30 pellucida. The viral vector system used to introduce the transgene is typically a replication-defective retrovirus carrying the transgene (Jahner *et al.*, (1985) Proc. Natl.

Acad. Sci. USA 82, 6927-6931; Van der Putten *et al.*, (1985) Proc. Natl. Acad. Sci. USA 82, 6148-6152). Transfection is easily and efficiently obtained by culturing the blastomeres on a monolayer of virus-producing cells (Van der Putten *et al.*, (1985) Proc. Natl. Acad. Sci. USA 82, 6148-6152; Stewart *et al.*, (1987) EMBO J. 6, 383-388).

- 5 Alternatively, infection can be performed at a later stage. Virus or virus-producing cells can be injected into the blastocoele (Jahner *et al.*, (1982) Nature 298, 623-628). Most of the founder animals will be mosaic for the transgene since incorporation occurs only in a subset of the cells which formed the transgenic non-human animal. Furthermore, the founder animal may contain retroviral insertions of the transgene at a variety of positions
- 10 in the genome; these generally segregate in the offspring. In addition, it is also possible to introduce transgenes into the germ line, albeit with low efficiency, by intrauterine retroviral infection of the midgestation embryo (Jahner *et al.*, (1982) Nature 298, 623-628).

- A third type of target cell for transgene introduction is the embryonal stem cell (ES). ES cells are obtained from pre-implantation embryos cultured *in vitro* (Evans *et al.*, (1981) Nature 292, 154-156; Bradley *et al.*, (1984) Nature 309, 255-256; Gossler *et al.*, (1986) Proc. Natl. Acad. Sci. USA 83, 9065-9069). Transgenes can be efficiently introduced into ES cells by DNA transfection or by retrovirus-mediated transduction. The resulting transformed ES cells can thereafter be combined with blastocysts from a
- 20 non-human animal. The ES cells colonize the embryo and contribute to the germ line of the resulting chimeric animal.

- The methods for evaluating the presence of the introduced DNA as well as its expression are readily available and well-known in the art. Such methods include, but are not limited to DNA (Southern) hybridization to detect the exogenous DNA, polymerase chain reaction (PCR), polyacrylamide gel electrophoresis (PAGE) and Western blots to
- 25 detect DNA, RNA and protein. The methods include immunological and histochemical techniques to detect expression of a gene.

- As used herein, a "transgene" is a DNA sequence introduced into the germline of a non-human animal by way of human intervention such as by way of the Examples described below. The nucleic acid sequence of the transgene, in this case a form of SEQ
- 30 ID NO: 66, may be integrated either at a locus of a genome where that particular nucleic

acid sequence is not otherwise normally found or at the normal locus for the transgene. The transgene may consist of nucleic acid sequences derived from the genome of the same species or of a different species than the species of the target animal.

As discussed above, a "vector" is any means for the transfer of a nucleic acid into a host cell. Preferred vectors are plasmids and viral vectors, such as retroviruses. Viral vectors may be used to produce a transgenic animal according to the invention. Preferably, the viral vectors are replication defective, that is, they are unable to replicate autonomously in the target cell. In general, the genome of the replication defective viral vectors which are used within the scope of the present invention lack at least one region which is necessary for the replication of the virus in the infected cell. These regions can either be eliminated (in whole or in part), or be rendered non-functional by any technique known to a person skilled in the art. These techniques include the total removal, substitution (by other sequences, in particular by the inserted nucleic acid), partial deletion or addition of one or more bases to an essential (for replication) region. Such techniques may be performed *in vitro* (on the isolated DNA) or *in situ*, using the techniques of genetic manipulation or by treatment with mutagenic agents.

Preferably, the replication defective virus retains the sequences of its genome which are necessary for encapsidating the viral particles. The retroviruses are integrating viruses which infect dividing cells. The retrovirus genome includes two LTRs, an encapsidation sequence and three coding regions (*gag*, *pol* and *env*). The construction of recombinant retroviral vectors has been described (see, for example, Bernstein *et al.*, (1985) Genet. Eng. 7, 235; McCormick, (1985) Biotechnol. 3, 689-691). In recombinant retroviral vectors, the *gag*, *pol* and *env* genes are generally deleted, in whole or in part, and replaced with a heterologous nucleic acid sequence of interest. These vectors can be constructed from different types of retrovirus, such as, HIV, MoMuLV (murine Moloney leukemia virus), MSV (murine Moloney sarcoma virus), HaSV (Harvey sarcoma virus); SNV (spleen necrosis virus); RSV (Rous sarcoma virus) and Friend virus.

In general, in order to construct recombinant retroviruses containing a nucleic acid sequence, a plasmid is constructed which contains the LTRs, the encapsidation sequence and the coding sequence. This construct is used to transfect a packaging cell line, which cell line is able to supply in trans the retroviral functions which are deficient in the



plasmid. In general, the packaging cell lines are thus able to express the *gag*, *pol* and *env* genes. Such packaging cell lines have been described in the prior art, in particular the cell line PA317 (U.S. Patent No. 4,861,719); the PsiCRIP cell line (WO9002806) and the GP+envAm-12 cell line (WO8907150). In addition, the recombinant retroviral vectors can contain modifications within the LTRs for suppressing transcriptional activity as well as extensive encapsidation sequences which may include a part of the *gag* gene (Bender *et al.*, (1987) J. Virol. 61, 1639-1646). Recombinant retroviral vectors are purified by standard techniques known to those having ordinary skill in the art.

In one aspect the nucleic acid encodes antisense RNA molecules. In this embodiment, the nucleic acid is operably linked to suitable regulatory regions (discussed above) enabling expression of the nucleic acid sequence, and is introduced into a cell utilizing, preferably, recombinant vector constructs, which will express the antisense nucleic acid once the vector is introduced into the cell. Examples of suitable vectors includes plasmids, adenoviruses, adeno-associated viruses (see, for example, U.S. Patent No. 4,797,368, U.S. Patent No. 5,139,941), retroviruses (see above), and herpes viruses. For delivery of a therapeutic gene the vector is preferably an adeno-associated virus.

Adenoviruses are eukaryotic DNA viruses that can be modified to efficiently deliver a nucleic acid of the invention to a variety of cell types. Various serotypes of adenovirus exist. Of these serotypes, preference is given, within the scope of the present invention, to using type two or type five human adenoviruses (Ad 2 or Ad 5) or adenoviruses of animal origin (see WO9426914). Those adenoviruses of animal origin which can be used within the scope of the present invention include adenoviruses of canine, bovine, murine, ovine, porcine, avian, and simian origin.

The replication defective recombinant adenoviruses according to the invention can be prepared by any technique known to the person skilled in the art. In particular, they can be prepared by homologous recombination between an adenovirus and a plasmid which carries, inter alia, the DNA sequence of interest. The homologous recombination is effected following cotransfection of the said adenovirus and plasmid into an appropriate cell line. The cell line which is employed should preferably (i) be transformable by the said elements, and (ii) contain the sequences which are able to complement the part of the genome of the replication defective adenovirus, preferably in integrated form in order to

avoid the risks of recombination. Recombinant adenoviruses are recovered and purified using standard molecular biological techniques, which are well known to one of ordinary skill in the art.

5 A number of recombinant or transgenic mice have been produced, including those which express an activated oncogene sequence (U.S. Patent No. 4,736,866); express Simian SV 40 T-antigen (U.S. Patent No. 5,728,915); lack the expression of interferon regulatory factor 1 (IRF-1) (U.S. Patent No. 5,731,490); exhibit dopaminergic dysfunction (U.S. Patent No. 5,723,719); express at least one human gene which participates in blood pressure control (U.S. Patent No. 5,731,489); display greater similarity to the conditions  
10 existing in naturally occurring Alzheimer's disease (U.S. Patent No. 5,720,936); have a reduced capacity to mediate cellular adhesion (U.S. Patent No. 5,602,307); possess a bovine growth hormone gene (Clutter *et al.*, (1996) Genetics 143, 1753-1760) or are capable of generating a fully human antibody response (Zou *et al.*, (1993) Science 262, 1271-1274).

15 While mice and rats remain the animals of choice for most transgenic experimentation, in some instances it is preferable or even necessary to use alternative animal species. Transgenic procedures have been successfully utilized in a variety of non-murine animals, including sheep, goats, chickens, hamsters, rabbits, cows and guinea pigs (see Aigner *et al.*, (1999) Biochem. Biophys. Res. Commun. 257, 843-850; Castro *et al.*,  
20 (1999) Genet. Anal. 15, 179-187; Brink *et al.*, (2000) Theriogenology 53, 139-148; Colman, (1999) Genet. Anal. 15, 167-173; Eyestone, (1999) Theriogenology 51, 509-517; Baguisi *et al.*, (1999) Nat. Biotechnol. 17, 456-461; Prather *et al.*, (1999) Theriogenology 51, 487-498; Pain *et al.*, (1999) Cells Tissues Organs 165, 212-219; Fernandez *et al.*, (1999) Indian J. Exp. Biol. 37, 1085-1092; U.S. Patent Nos. 5,908,969; 5,792,902;  
25 5,892,070; 6,025,540).

The practice of the present invention will employ the conventional terms and techniques of molecular biology, pharmacology, immunology and biochemistry that are within the ordinary skill of those in the art. For example, see Sambrook *et al.*, (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press.

30 Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize

the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

5

## EXAMPLES

### Example 1 - Recombinant proteins, fusion proteins and protein tags

Cyclin E, p27, SKP1, and SKP2 were each cloned into pVL1392 (PharMingen) vector as glutathione-S-transferase (GST) fusion proteins. In addition to GST fusion  
10 proteins, SKP2 or SKP2-like proteins could be fused with a protein interaction domain such as Max, which binds to c-Myc, to target c-Myc for ubiquitination and degradation in cells. Human CUL-1, SKP2 and SKP1 cDNA were also cloned directly into baculovirus pVL1392 or pVL1393 expression vectors. The construction of these baculoviruses was accomplished as previously described (Zhang *et al.*, (1995) Cell 82, 915-925). The  
15 baculoviruses for CDK2 and GST-cyclin A were also constructed as previously described (Zhang *et al.*, (1995) Cell 82, 915-925). The cDNA clone encoding human E1 ubiquitin was cloned into the baculovirus expression vector, pAcSG-His-NT (PharMingen), as a histidine<sub>6</sub> tagged protein. In addition, SKP2 or SKP2-like proteins could be tagged with a protein interaction domain such as Max, which binds to c-Myc, to target c-Myc for  
20 ubiquitination and degradation in cells.

The E1 protein was expressed in the baculovirus expression system and purified by ubiquitin affinity chromatography (Yu *et al.*, (1998) Proc. Natl. Acad. Sci. USA 95, 11324-11329). The purification was monitored by protein staining and the E1 activity was assayed by covalent conjugation of biotinylated ubiquitin (Pagano *et al.*, (1995) Science  
25 269, 682-685). For <sup>35</sup>S- labeled p27, SF9 cells were infected with baculoviruses encoding GST-p27. Forty hours post-infection, cells were labeled with <sup>35</sup>S-methionine for three hours as described (Zhang *et al.*, (1995) Cell 82, 915-925). The labeled GST-p27 protein was isolated by glutathione Sepharose beads and the p27 portion was released from the beads by thrombin treatment for thirty minutes at room temperature (Calbiochem) (Guan  
30 *et al.*, (1991) Anal. Biochem. 192, 262-267). Thrombin was subsequently inactivated by

one mM phenylmethyl-sulfonyl fluoride (PMSF). The purified p27 is monitored by autoradiography and quantified by protein staining and Western-blot analysis.

To assemble cyclin E/CDK2, GST-cyclin E and CDK2 baculoviruses were individually expressed in SF9 cells. The lysates were prepared in hypotonic buffer (20 mM Hepes, pH 7.2, 5 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.5 mM DTT). The lysates containing GST-cyclin E and CDK2 were mixed and incubated in the presence of 10 mM ATP at 30 °C for one hour to assemble the active cyclin E/CDK2 kinase. The kinase was then affinity purified using the glutathione beads and quantified by protein staining and Western blot. The activity of purified kinase was monitored by the histone H1 assay (Zhang *et al.*, (1995) Cell 82, 915-925). To produce SCF<sup>SKP2</sup> complex, baculoviruses encoding GST-SKP1 and CUL-1, in the presence or absence of baculoviruses encoding SKP2, were co-infected into insect SF9 cells and were affinity purified using a glutathione Sepharose column. The successful assembly of the complex was monitored and quantified by protein staining and Western-blot analysis (Figure 15). The *in vitro* translated proteins were produced and labeled with <sup>35</sup>S-methionine in TNT rabbit reticulocyte lysates according to the manufacturer's instructions (Promega).

The human CDC34 cDNA clone was cloned into pGEXKG as a GST fusion protein and expressed in bacteria BL21. GST-CDC34 was isolated by glutathione column and the GST portion was removed by thrombin. The CDC34 protein was further purified with a MonoQ column and monitored by protein staining. The methyl ubiquitin and ubiquitin aldehyde were commercially obtained (BostonBiochem).

Anti-p27 (sc-528) antibodies were purchased commercially (Santa Cruz Biotechnology). Rabbit anti-cyclin E, SKP2, and CDK2 polyclonal antibodies and anti-HA epitope tag monoclonal antibody (12CA5) were described previously (Zhang *et al.*, (1995) Cell 82, 915-925; Xiong *et al.*, (1993) Nature 366, 701-714). For some experiments, a monoclonal anti-human cyclin E antibody (HE12) and a polyclonal anti-mouse cyclin E antibody (M20) were used (Santa Cruz). The anti-T7-tag monoclonal antibody was obtained from Novagen. Immunoprecipitation and Western-blot analyses were performed as described previously (Zhang *et al.*, (1995) Cell 82, 915-925). For direct Western-blotting, cells were lysed directly in 0.1% SDS, and viscosity was reduced by passing the lysates through a 22-gauge needle. Approximately 40 µg of proteins were

loaded directly onto an SDS-polyacrylamide gel for Western-blot analysis. Identical results were obtained from direct Western-blot analyses as from immunoprecipitation followed by Western-blot analyses.

5     Example 2 - Phosphorylation-dependent p27 degradation

          Selective p27 degradation in cell free systems has been reported previously in synchronized S-phase extracts but not in G1 cell extracts (Nguyen *et al.*, (1999) Mol. Cell. Biol. 19, 1190-1201; Brandeis & Hunt (1995) EMBO J. 15, 5280-5289). To determine the proteins that control p27 stability, cytosolic extracts from asynchronized and exponentially  
10   growing HeLa cells were prepared.

          HeLa cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum at 37°C. For extract preparation, suspension HeLa cells were grown to  $0.5-1 \times 10^6$  cells/ml (log-phase) and extracts were prepared as previously described (Brandeis & Hunt (1996) EMBO J. 15, 5280-5289). Cell pellets  
15   were washed twice with phosphate-buffered saline (PBS) and then with hypotonic buffer. The cells were re-suspended in two volumes of hypotonic buffer. They were lysed by Dounce homogenization using a loose pestle. The cytosolic extracts were prepared by centrifugation at 15,000 rpm using a Sorvall SS34 rotor. Aliquots of the extracts were immediately frozen in liquid nitrogen and stored at -80°C.

20           For a typical degradation reaction of p27, 200 µg cytosolic extract was used in a total volume of 50 µl, with no greater than 20% dilution of the extract. The reaction mixture also contained 2 mM ATP, 20 mM creatine phosphate, 50 µg/ml creatine kinase, 20 mM Hepes, pH7.2, 1 mM DTT, and 10 mM MgCl<sub>2</sub>. The reactions were initiated by adding <sup>35</sup>S-labeled p27 (0.25-0.5 µg) and cyclin E/CDK2 (1-3 µg) and incubation at 30°C  
25   for one to three hours. The amount of cyclin E/CDK2 required for p27 degradation was titrated batchwise for different extract preparations to determine the necessary threshold level of cyclin E/CDK2. The requirement of cyclin E/CDK2 is also dependent on the amount of exogenously added p27, reflecting the fact that p27 serves both as an inhibitor and a substrate for the kinase. Substantial amounts of endogenous p27 were present in the  
30   extract which was also degraded by addition of cyclin E/CDK2. The reactions were stopped by adding 0.1% SDS, followed by one ml of lysis buffer and in the presence of

protease inhibitors (5 µg/ml of leupeptide, soybean trypsin inhibitor and aprotinin plus 100 mM benzamidine). The reaction products were immunoprecipitated using p27 antibodies, fractionated in SDS-PAGE, and visualized by autoradiography. Degradation of endogenous p27 in the extracts was monitored by directly loading onto an SDS-PAGE in the SDS sample buffer, followed by Western-blotting with p27 antibodies.

Recombinant <sup>35</sup>S-labeled p27 was not degraded when incubated with this cytosolic extract indicating that p27 was quite stable. Addition of an active cyclin E/CDK2 kinase to the extract led to the rapid degradation of p27 (Figures 1A, 1B). The requirement of cyclin E/CDK2 for p27 destruction was due to its ability to phosphorylate p27 at threonine 187, which has been shown to trigger p27 degradation (Sheaff *et al.*, (1997) Genes Dev. 11, 1464-78; Vlach *et al.*, (1997) EMBO J. 16, 5334-44). Conversion of threonine 187 to glycine (T187G) stabilized p27 in the extract and confirmed the requirement for phosphorylation of threonine 187 (Figure 1A). In the presence of cyclin E/CDK2, a fraction of p27 slightly shifted its electrophoretic mobility which was sensitive to phosphatase (Figure 1C), indicating that these proteins are phosphorylated forms of p27.

A fraction of p27 was converted into multiple and high molecular weight species in the presence of cyclin E/CDK2 (Figure 1C), which are insensitive to phosphatase treatment. Addition of specific inhibitors of 26S proteasome, such as MG132, stabilized p27 and resulted in accumulation of both the phosphorylated and the high molecular weight forms of p27 (Figure 1C). Modified ubiquitins such as methyl ubiquitin and ubiquitin aldehyde also caused accumulation of p27 ladders (Figure 2) due to their ability to interfere with the degradation rate or inhibition of deubiquitination of highly polyubiquitinated proteins (Hershko *et al.*, (1987) Proc. Natl. Acad. Sci. USA 84, 1829-1833). These observations indicate that the high molecular weight species of p27 are the poly-ubiquitinated. This *in vitro* system therefore faithfully recapitulated the ubiquitin-dependent p27 degradation in a cyclin E/CDK2-dependent process which requires phosphorylation of the threonine 187 residue on p27.

### Example 3 - Alteration of p27 levels by depletion of SKP1, SKP2 & CUL-1

Using the *in vitro* p27 degradation system, the potential involvement of candidate ubiquitin E3 ligases, the SCF complexes (SKP1, CDC53/Cullins, F-box proteins), for p27

degradation was examined. The SCF complexes represent a conserved family of protein complexes that target phosphorylated proteins for ubiquitin-dependent proteolysis (Patton *et al.*, (1998) Trends Genet. 14, 236-243; Maniatis, (1999) Gene Dev. 13, 505-510).

CUL-1 was examined first to determine whether a human CDC53 homologue is necessary for p27 degradation.

For depletion of CUL-1, SKP1 or SKP2 proteins from HeLa extracts, four mg of affinity purified CUL-1, SKP1 or SKP2 antibodies (Zhang *et al.*, (1995) Cell 82, 915-925) or IgG were coupled to one ml protein A-Sepharose column. Five to ten ml of HeLa extracts were used to pass through the antibody-protein A column three times at 4 °C. The flow-through fractions from the columns were collected and examined for the efficiency of depletion using Western blot analysis. These fractions were then used as depleted extracts. For restoration of p27 degradation activity in SKP2 depleted extract, two µg of purified SKP2, SCF<sup>SKP2</sup> or SC (no SKP2) complexes were added to SKP2 depleted extracts and the degradation of p27 was monitored as described above.

Depletion of CUL-1 abolished the ability of the extracts to degrade p27 while parallel mock depletion using purified IgG from the pre-immune serum had no effect (Figure 3A). Western-blot analysis confirmed that CUL-1 was removed from the extracts by the column (Figure 2D). Specific immuno-depletion of another component of the SCF complex, SKP1, also resulted in the inhibition of p27 degradation which led to an increase in p27 levels (Figures 3B, 3C).

The requirement for CUL-1 and SKP1 for p27 degradation implies that an F-box protein is involved. The F-box protein is a component in the SCF complexes that interacts directly with the phosphorylated substrates and thus defines the substrate specificity for ubiquitination (Maniatis, (1999) Genes Dev. 13, 505-510; Skowyra *et al.*, (1997) Cell 91, 209-219; Winston *et al.*, (1999) Genes Dev. 13, 270-83). To identify the F-box protein(s) that specifically bind to p27, <sup>35</sup>S-labeled HeLa cell extracts were incubated with GST-p27 either with or without prior phosphorylation by cyclin E/CDK2. Examination of the labeled proteins specifically associated with the phosphorylated GST-p27 beads revealed the presence of a 45 kDa protein, which is similar to the molecular weight of the F-box protein SKP2.

To determine whether SKP2 is involved in p27 degradation, HeLa extracts were subjected to immuno-depletion with an affinity purified SKP2 antibody column. Removal of SKP2 by immunodepletion of SKP2 from the extract resulted in the inhibition of p27 degradation activity in the extract (Figure 3C, 3D). This data in combination with the SKP1 depletion experiments indicates that depletion of SKP proteins results in modulation of SKP activity which can increase expression of p27.

#### Example 4 - SKP2 binds to phosphorylated p27

To directly examine the specific binding of SKP2 to the phosphorylated form of p27, a pair of peptides corresponding to the carboxy-terminal end of p27 (amino acids 175-198) was synthesized. Threonine 187 was phosphorylated in the first peptide but not in the second peptide (Figure 4A). The peptides were each coupled to SulfoLink agarose beads which were then used as affinity resins for binding analysis of F-box proteins. These peptides were initially tested to determine if they could interact with several known F-box proteins, including SKP2,  $\beta$ -TrCP and MD6, as well as a number of unpublished F-box proteins identified through EST database search. *in vitro* translated and  $^{35}\text{S}$ -labeled F-box proteins were incubated with the p27 peptide beads. Analysis of the F-box proteins associated with p27 peptide beads revealed a specific interaction between SKP2 and the phosphorylated threonine 187 p27 peptide (Figure 4B). No significant interactions were observed if the non-phosphorylated form of the peptide was used. Specific associations between the p27 phosphopeptide and other available F-box proteins were not detected (Figure 4B, data not shown). These data indicate that SKP2 can interact selectively and specifically with the p27 phosphopeptide.

To determine whether endogenous SKP2 in the HeLa extract can also interact with p27 phosphopeptide, the peptide beads were incubated with the extracts. The peptides, containing either the carboxy-terminal end of p27 (amino acids 175-198), CSDGSPNAGSVEQTPKKPGLRRRQT, and phosphopeptides CSDGSPNAGSVEQ\*TPKKPGLRRRQT (\*T denotes phosphorylated threonine 187 of p27) (SEQ ID NO: 1) were synthesized using the peptide synthesis facility at the Yale University School of Medicine. The phosphorylated threonine 187 (\*T) and the non-phosphorylated forms of the p27 carboxy-terminal peptides were conjugated to



SulfoLink beads (Pierce) through the cysteine residue added at the amino-terminus of the peptides according to manufacturer's instruction (Pierce). For coupling reactions, 0.5 mg of peptides were conjugated onto two ml of Sulfolink beads for thirty minutes and the residue sites on the beads was blocked by 20 mM cysteine for two hours at room

5 temperature. The beads were washed extensively first with PBS followed by hypotonic buffer and stored at 4 °C. For F-box protein binding assays, 10 µl of the *in vitro* translated F-box proteins, including SKP2, β-TrCP, and MD6, were mixed 20 µl peptide beads in 250 µl of lysis buffer containing protease inhibitors (5 µg/ml leupeptide, trypsin inhibitor, aprotinin, and 100 mM benzamidine) and 100 mM NaF. Binding assays were performed  
10 at 4 °C for one hour with agitation. The beads were washed with detergent buffer for four times and the proteins associated with the beads were analyzed. A similar procedure was used for the extract binding except 100-400 µg of HeLa extracts were used as the source of SCF complexes, replacing the *in vitro* translated F-box proteins.

Western-blot analysis detected a strong and specific interaction between  
15 endogenous SKP2 and the p27 phosphopeptide (Figure 4C). The p27 peptide without threonine 187 phosphorylation did not significantly interact with SKP2. As a control for SKP2 binding, binding of β-TrCP to the p27 peptides was also measured. β-TrCP is an F-box protein that binds and targets phosphorylated β-catenin and IB for ubiquitin-dependent degradation (Maniatis, (1999) Genes Dev. 13, 505-510). The data  
20 indicated that although β-TrCP was also present in the extract, no interactions between β-TrCP and the p27 phosphopeptide were detected in these assays (Figure 4C). These studies confirm that endogenous SKP2 in the HeLa extract specifically recognizes and binds to the phosphorylated form of p27. In addition to SKP2 binding only to the phosphorylated form p27 peptide, it also binds only to the phosphorylated form of cyclin E  
25 peptide SPLPSGLL\*TPPQSGKKQSSGPEMA (amino acids 372-395 where \*T denotes phosphorylated threonine 187 of p27) (SEQ ID NO: 4). SKP2 can therefore be inhibited by a phosphopeptide other than p27 phosphopeptide.

Previous studies indicated that SKP2 interacts with SKP1 and CUL-1 *in vivo* (Yu *et al.*, (1998) Proc. Natl. Acad. Sci. USA 95, 11324-11329; Lisztwan *et al.*, (1998) EMBO  
30 J. 17, 368-83; Lyapina *et al.*, (1998) Proc. Natl. Acad. Sci. USA 95, 7451-7456; Michel *et al.*, (1998) Cell Growth Differ. 9, 435-449). SKP2 interactions with SKP1 and CUL-1

were also observed in the HeLa extracts (Figure 4D). It was necessary to confirm that SKP1 and CUL-1 could interact directly with the p27 since depletion of either of these proteins from the extracts also abolished p27 degradation. Confirmation of such a direct interaction would eliminate the possibility that other indirect mechanisms were responsible for the increase in p27 following removal of SKP1 or CUL-1. Using the peptide bead pull-down assays, specific interactions of SKP1 or CUL-1 with the phosphorylated threonine p27 peptide beads were detected in contrast to the non-phosphorylated peptide where no such interaction was detected (Figure 4C). To determine whether SKP2 mediates SKP1 and CUL-1 binding to p27 phosphopeptide, SKP1 or CUL-1 binding in SKP2 depleted extracts was examined. Depletion of SKP2 from the extract significantly reduced the binding of SKP1 (Figure 4E) or CUL-1 (data not shown) to the p27 phosphopeptide beads, although the total levels of SKP1 and CUL-1 in the extract were not substantially altered by SKP2 depletion (Figure 4E and data not shown). These studies indicate that SKP2 is the SCF component that binds to the phosphorylated threonine 187 of p27. Upon SKP2 binding to phosphorylated p27, SKP2 associates with SKP1 and CUL-1 and targets p27 for ubiquitin-dependent degradation by the 26S proteasome. Modulation of the activity of SKP2 can therefore increase the levels of p27.

#### Example 5 - SKP2-dependent degradation of p27

The effect of addition of the SCF<sup>SKP2</sup> complex to the SKP2 depleted extract was investigated to determine if restoration of SKP2 is sufficient to restore p27 degradation activity. Recombinant SCF<sup>SKP2</sup> complexes were expressed, assembled using the baculovirus expression system and purified. When the recombinant SCF<sup>SKP2</sup> complex was added back into the SKP2 depleted extract, restoration of p27 degradation was observed (Figure 5A). Restoration of p27 degradation was dependent on the presence of SKP2 in the complex because complexes assembled in the absence of SKP2 could not rescue the SKP2 deficiency in the extract. Addition of purified SKP2 alone could partially rescue p27 degradation in the SKP2 depleted extract (data not shown) but the assembled SCF<sup>SKP2</sup> complex consistently produced better restoration, indicating that the SCF<sup>SKP2</sup> complex itself is required for p27 degradation. When SKP2 expression is under the control of a tetracycline-inducible promotor in HeLa cells, removal of tetracycline results in expression

of SKP2. Induction of SKP2 resulted in approximately a significant decrease in total cellular p27. Since SKP2 only targets the Thr187-phosphorylated p27 for degradation, the down-regulation of p27 by expressing SKP2 indicates that SKP2 is rate limiting where sufficient CUL-1 and SKP-2 are present.

5

#### Example 6 - SKP2-dependent ubiquitination of p27

p27 ubiquitination was also assayed directly using the recombinant SCF<sup>SKP2</sup> complex. In a purified system containing the recombinant SCF<sup>SKP2</sup> complex, cyclin E/CDK2, ubiquitin activation enzyme E1, and ATP, a fraction of p27 was converted into multiple high molecular weight species (Figure 5B). The formation of high molecular weight p27 was dependent on the presence of ubiquitin and CDC34, a conserved E2 conjugating enzyme that is implicated in SCF-mediated ubiquitination (King *et al.*, (1996) Science 274, 1652-1659; Plon *et al.*, (1993) Proc. Natl. Acad. Sci. USA 90, 10484-10488). The E2 conjugating enzyme for SCF<sup>SKP2</sup> is probably a human CDC34 homolog. These data suggest that the SCF<sup>SKP2</sup> complex can ubiquitinate p27 in the presence of E1 and E2. However, p27 ubiquitination using the purified proteins was not very efficient. It is possible that SCF<sup>SKP2</sup> may require additional modifications or activities for efficient p27 ubiquitination.

#### 20 Example 7 - SKP2 binds to phosphorylated cyclin E

N-acetyl-L-leuciny-L-leucinal-L-norleucinal (LLNL) and hydroxyurea (HU) were purchased from Sigma. The cyclin E carboxy peptides (residues 371-394) CASPLPSGLLTTPQSGKKQSSGPEM containing either the Thr380-phosphorylated (TP-CP) or non-phosphorylated (TP-C) forms were synthesized and coupled to Sulfo-Link agarose beads (Pierce) as described previously (Tsvetkov *et al.*, (1999) Curr. Biol. 9, 661-664) (TP: corresponding to Thr380 and Pro381 in cyclin E). Cyclin E mutant peptides, TA-CP (CASPLPSGLLTAPQSGKKQSSGPEM) (SEQ ID NO: 5), SP-C and SP-CP (CASPLPSGLLSPPQSGKKQSSGPEM) (SEQ ID NO: 6), were synthesized accordingly. A cysteine residue was added to the amino-terminal end of these peptides to facilitate coupling to the beads. The cyclin E cDNA was tagged by the T7-epitope tag at its amino-terminus in pCGT, and its expression was under CMV promoter control. The SKP2

dominant negative mutant (SKP2DN) lacking the F-box was constructed as described (Carrano *et al.*, (1999) Nat. Cell. Biol. 1, 193-199). Both the wild-type and mutant SKP2 were cloned into the retrovirus vector pBabe. The full-length cDNA clones of FBL-2, -5, -6 and -8 were commercially purchased (Research Genetics) and were sequenced for confirmation.

To identify the F-box protein(s) that might bind to the phosphorylated Thr380 in cyclin E, a pair of peptides that correspond to the carboxy-terminal end of cyclin E that includes the critical Thr380 (Figure 6A) were synthesized. One peptide (TP-CP) contained the phosphorylated Thr380, and the other had a nonphosphorylated Thr380 (TP-C). Each of these peptides was immobilized onto agarose beads, which were then used to determine the binding of various F-box proteins. The F-box proteins were *in vitro* translated and <sup>35</sup>S-methionine-labeled. They were used directly to test for binding to TP-CP or TP-C beads. Using this assay, it was determined that SKP2 selectively interacted with the cyclin E phosphopeptide TP-CP, while no detectable interactions were observed between SKP2 and the nonphosphorylated cyclin E peptide TP-C. The interaction between the phosphorylated cyclin E peptide and SKP2 is specific, since it was not possible to detect the binding of cyclin E TP-CP to other F-box proteins, including various FBLs (Winston *et al.*, (1999) Curr. Biol. 9, 1180-1182; Cenciarelli *et al.*, (1999) Curr. Biol. 9, 1177-9), which bears close homologies to SKP2 as well as the more distantly related  $\beta$ -TrCP and MD6 (Figure 6B and data not shown).

SKP2 normally forms a complex with SKP1 and CUL-1 (Tsvetkov *et al.*, (1999) Curr. Biol. 9, 661-664). To determine whether the SCF<sup>SKP2</sup> complex binds specifically to the phosphorylated cyclin E peptide, a cytosolic HeLa cell extract was used as the source of SCF complexes (Figure 6C). Thus, SKP2, SKP1, and CUL-1 all interact specifically with the cyclin E phosphopeptide TP-CP but not with the nonphosphorylated cognate peptide TP-C (Figure 6C). To rule out the possibility that SKP2 nonspecifically binds to phosphorylated peptides, a number of mutant peptide derivatives were synthesized in which either Thr380 in cyclin E was converted into serine or phosphoserine (SP-C or SP-CP) or Pro381 was converted into alanine but with Thr380 remaining phosphorylated (TA-CP). Binding assays indicate that SKP2 did not interact with the mutant SP-CP and TA-CP phosphopeptides. Similar results were obtained in a peptide competition experiment in

which increasing amounts of either TP-CP, TA-CP, or SP-CP phosphopeptides were used as competitors for the association between SKP2 and the TP-CP beads (Figure 6D).

Example 8 - Phosphorylation-dependent cyclin E degradation

5 SKP2 expression is periodic in a cell-cycle-dependent manner, with a peak level in the S phase (Zhang *et al.*, (1995) Cell 82, 915-925). Recent evidence suggests that SKP2 is a limiting component of the SCF<sup>SKP2</sup> complex for S phase entry and for the degradation of p27 (Sutterluty *et al.*, (1999) Nat. Cell Biol. 1, 207-214; Tsvetkov *et al.*, (1999) Curr. Biol. 9, 661-664; Carrano *et al.*, (1999) Nat. Cell. Biol. 1, 193-199; Zhang *et al.*, (1995) Cell 82, 915-925). To determine whether cyclin E is a target for ubiquitination by SKP2, the levels of T7-epitope-tagged cyclin E were examined after its transfection into HeLa cells in the presence or absence of SKP2. SKP2 expression caused a substantial reduction in the levels of co-expressed cyclin E. This effect is dependent on the Thr380 residue in cyclin E. When Thr380 was converted into glycine (T380G), which could not be phosphorylated, the mutant cyclin E was much more resistant to SKP2 (Figure 7A). Pulse-and-chase experiments indicated that SKP2 significantly shortened the half-life of the cyclin E protein (Figure 8A).

In addition, expression of SKP2 induced the formation of high-molecular-weight ladders of cyclin E (Figure 7B-E) in both 293 and mouse embryonic fibroblast cells. The SKP2-dependent formation of high-molecular-weight ladders of cyclin E was mostly abolished if Thr380 of cyclin E was mutated into glycine (T380G). To determine whether the high-molecular-weight species were polyubiquitinated forms of cyclin E, the effect of expressing an HA-tagged ubiquitin (HAUb) on cyclin E was examined. Expression of HAUb also led to the accumulation of the high-molecular-weight forms of cyclin E similar to the ones induced by SKP2 (Figure 7C). Immunoprecipitation with anti-HA epitope antibody followed by Western-blotting with T7-tagged cyclin E revealed that the high-molecular-weight species of cyclin E were polyubiquitinated forms of cyclin E (Figure 7D).

Furthermore, expression of SKP2 greatly promoted high levels of incorporation of HAUb into cyclin E, as compared with that of HAUb alone (Figure 7D). These observations indicate that expression of SKP2 is sufficient to cause the polyubiquitination

of cyclin E *in vivo*. In addition, the effect of T380G mutation in cyclin E on the polyubiquitination of cyclin E indicates that ubiquitination is dependent on the presence of Thr380. However, in the absence of Thr380, a weaker but detectable level of cyclin E ubiquitination was observed (Figure 7B). This ubiquitination was also promoted by SKP2. Although the phosphorylated Thr380 provides a major binding site for SKP2, there exists additional minor sites in cyclin E that can be used for SKP2 binding and cyclin E ubiquitination.

Example 9 - SKP2-dependent ubiquitination of cyclin E independent of p27

The F-box proteins usually interact directly with their phosphorylated substrates. To determine the potential association of SKP2 with full-length cyclin E, T7-tagged wild-type or the T380G mutant form of cyclin E was expressed in the presence or in the absence of N-acetyl-L-leuciny-L-leucinal-L-norleucinal (LLNL), a specific inhibitor of the 26S proteasome *in vivo* (Figure 8B). Immunoprecipitation followed by Western-blotting indicated that both un-ubiquitinated and ubiquitinated forms of cyclin E were associated with SKP2 (Figure 8B). The cyclin E T380G mutant was also found to be associated with SKP2 (Fig. 3B) but to a lesser extent. This is consistent with the earlier observation (Figure 7B) that the phosphorylated Thr380 is a major site for SKP2 binding but that there are additional minor sites in cyclin E for SKP2 binding and ubiquitination. Since cyclin E-SKP2 interaction (Figure 8B) and ubiquitination (Figure 7E) occurred in p27<sup>-/-</sup> mouse embryonic cells, this indicates that cyclin E ubiquitination (Figure 7E and 8B) and its interaction with SKP2 (Figure 8B) are independent of p27.

Although cyclin E ubiquitination is independent of p27, in the presence of co-expressed CDK inhibitor p27, cyclin E degradation was inhibited even in the presence of SKP2 (Figure 8C). This observation indicates that p27 might inhibit cyclin E autophosphorylation on Thr380, leading to resistance to SKP2-mediated ubiquitin-dependent degradation of cyclin E. The effect of p27 is not to be due to a competition between p27 and cyclin E for SKP2 binding, since a non-phosphorylated mutant form of p27 in which the critical Thr187 was converted into glycine (T187G) did not bind. This data is consistent with the previous report that p27 inhibits the Thr380-dependent cyclin E degradation (Clurman *et al.*, (1996) Genes Devel. 10, 1979-1990).

Expression of SKP2 also affects the endogenous cyclin E level. When SKP2 was expressed in cells using recombinant retrovirus delivery system, a significant decrease in endogenous cyclin E levels was observed (Figure 9A and B). As observed before, ectopic expression of SKP2 also led to the reduction of p27 levels. The possibility that cyclin E down-regulation is due to a secondary effect of SKP2 on the S phase was eliminated since SKP2 caused the decrease of cyclin E even in cells that were synchronized in the S phase by hydroxyurea (Figure 7B). Conversely, expression of a dominant-negative SKP2 (DN) that is defective in F-box, a binding site for SKP1, caused the accumulation of endogenous cyclin E (Figure 8C and D). Such an effect on the endogenous cyclin E is independent of p27, since SKP2DN-mediated elevation of cyclin E could occur in p27<sup>-/-</sup> mouse embryonic fibroblasts (Figure 8D). This observation is consistent with our finding that SKP2-mediated-ubiquitination of cyclin E occurs in p27<sup>-/-</sup> mouse embryonic fibroblasts (Figure 7E). This data indicates that SKP2-mediated cyclin E ubiquitination is p27-independent.

Applicants have identified SKP2 as an F-box protein that mediates ubiquitin-dependent degradation of cyclin E. SKP2 is an F-box protein that is expressed in late G1, S, and G2 phases, playing a role in S phase of the cell cycle (Zhang *et al.*, (1995) Cell 82, 915-925). SCF<sup>SKP2</sup> binds and targets the CDK inhibitor p27 for ubiquitin-dependent degradation. In addition, SKP2 also interacts with cyclin E and plays a role in the ubiquitin-dependent degradation of cyclin E. This SKP2-mediated cyclin E ubiquitination and degradation is mostly dependent on the presence of Thr380 in cyclin E (Figure 14), although weak cyclin E ubiquitination in the absence of Thr380 was also promoted by SKP2 *in vivo*.

Applicants have also identified that SKP2 performs a dual function during the G1/S transition. It is required for the ubiquitin-dependent degradation of p27 in late G1 (Sutterluty *et al.*, (1999) Nat. Cell. Biol. 1, 207-14; Tsvetkov *et al.*, (1999) Curr. Biol. 9, 661-664; Carrano *et al.*, (1999) Nat. Cell. Biol. 1, 193-199). The degradation of p27 by SCF<sup>SKP2</sup> activates cyclin E/CDK2 and promotes entry into the S-phase (Sutterluty *et al.*, (1999) Nat. Cell. Biol. 1, 207-14; Coats *et al.*, (1996) Science 272, 877-880). Once cells are in the S phase, cyclin E is degraded which may be required for terminating the S-phase initiation events, allowing the cells to progress from the S phase into the G2 phase

(Clurman *et al.*, (1996) Genes Dev. 10, 1979-1990; Won *et al.*, (1996) EMBO J. 15, 4182-4193).

Applicants have determined that a number of phosphorylation dependent and ubiquitin-dependent degradation events occur during the G1/S transition, which are temporally regulated. The expression of SKP2 in the late G1 and S phases leads to assembly of the SCF<sup>SKP2</sup> complex. Previous reports suggest that the phosphorylation status of p27 and cyclin E could be temporally separated. p27 phosphorylation on the critical Thr187 has been shown to occur in the late G1 phase and p27 ubiquitination has been reported to require its binding to the cyclin E/CDK2 complex (Montagnoli *et al.*, (1999) Genes Dev. 13, 1181-1189). The phosphorylation of Thr187 in p27 triggers the binding of SKP2, leading to the subsequent ubiquitin-dependent degradation of p27.

It has been shown that binding of p27 to cyclin E/CDK complexes inhibits the activity of cyclin E/CDK2 and cyclin E degradation (Clurman *et al.*, (1996) Genes Dev. 10, 1979-1990). The binding of p27 therefore prevents phosphorylation on Thr380 in cyclin E or there is a competition between p27 and cyclin E for the binding of SKP2. p27 binding can also cause a conformational change in cyclin E so that Thr380 in cyclin E is not exposed for phosphorylation or SKP2 binding. Applicants have determined that SKP2 binds to the p27 phosphopeptide with higher affinity than that of cyclin E peptide (data not shown). Thus the affinities between SKP2 and p27 or cyclin E may also affect the ubiquitination rate of p27 and cyclin E by SKP2. Once p27 is degraded, the cyclin E/CDK2 kinase activity is activated, leading to the S-phase entry. Activation of cyclin E also leads to its autophosphorylation in Thr380 (Clurman *et al.*, (1996) Genes Dev. 10, 1979-1990; Won & Reed, (1996) EMBO J. 15, 4182-4193). The phosphorylation of Thr380 promotes the SKP2 binding which in turn results in the ubiquitin-dependent degradation of cyclin E.

The efficiency of the ubiquitination reaction by the SCF complexes is very high. Based on the *in vitro* and *in vivo* p27 and cyclin E degradation using SKP2, the reaction efficiency can be 80-90% or even higher to 100% (Figure 14). This is a low estimation, since SKP2 only binds to phosphorylated substrates, the complete reaction is thus dependent on the extent of the substrate phosphorylation, which in turn relies on activities of kinases and phosphatase that regulate the levels of substrate phosphorylation *in vivo* or



in the cell extracts. Conversely, using the p27 phosphopeptide, it is possible to deplete almost all SKP2 in the cell extract. This indicates that SKP2 can bind to its substrates with very high affinity.

5     **Example 10 - SKP2 Fusion Proteins Capable of Altering Substrate Specificity**

          The substrate-specificity of SCF complexes can be altered if the substrate-binding domains of the F-box protein such as LRR in SKP2 or WD repeats in  $\beta$ -TRCP are replaced by other protein-protein interaction motifs. As a first test for such a possibility, a hybrid protein that contains the amino-terminus of  $\beta$ -TRCP up to its F-box motif was  
10     created (residues 1-204, the F-box is located between residues 148-191). However, the substrate-targeting domain of the WD repeats is replaced by the LRR region of SKP2 (residues 169-435, the F-box is between residues 112-151) (Figure 10). Such a fusion creates a hybrid protein ( $\beta$ -TRCP.N/SKP2.C) that contains the F-box region of  $\beta$ -TRCP and the SKP2 substrate-binding domain (leucine-rich repeats or LRR). This hybrid  $\beta$ -  
15     TRCP.N/SKP2.C protein would be expected to have an altered substrate specificity. Instead of normally targeting  $\beta$ -catenin and I $\kappa$ B by  $\beta$ -TRCP, the hybrid protein should target SKP2-specific substrates, such as cyclin E or p27, for ubiquitination and degradation. As expected, when this fusion protein is introduced into 293 cells, it is fully active to ubiquitinate cyclin E for polyubiquitination in the same way as SKP2 (Figure  
20     11). Thus, swapping the domain of F-box proteins can alter the substrate specificity of F-box proteins.

          By fusing the amino-terminus of  $\beta$ -TRCP and the carboxy terminus of SKP2, a fully active hybrid  $\beta$ -TRCP.N/SKP2.C protein was produced to target cyclin E for ubiquitination. The results from the  $\beta$ -TRCP.N/SKP2.C hybrid protein suggest that  
25     alteration of specificity of the F-box proteins can be made. However, it could not be ruled out that the LRR region of SKP2 contains a motif that is also required for SKP1 binding or the SCF ubiquitination activity.

          Sequence comparison (Figure 13) has revealed the presence of a relatively conserved motif at the carboxy-terminal region of SKP2 (residues 321-374) and  $\beta$ -TRCP  
30     (residues 429-497). This motif is also present in the yeast F-box CDC4 carboxy-terminus (residues 388-463). For convenience this domain will be designated SCM for SKP2 C-

terminal motif. The role of this motif is to mediate the interaction between the F-box proteins and SKP1 or other components of the SCF complexes. This possibility is based on our finding that von Hippel-Lindau disease protein ("VHL"), a human tumor suppressor protein that binds to a SKP1-like protein, elongin C/SIII in a putative SCF-like ubiquitin E3 ligase CUL-2/elongin B/C complex, also has this domain (residues 146-195) (Stebbins *et al.*, (1999) Science 284, 455-461). Fusing the F-box, a protein interaction domain, such as Max or the MDM2 amino-terminus, and this conserved SCM domain, should improve the ubiquitination of targeted protein by various hybrid proteins.

The VHL binds to CUL-2 and Elongin C (also called SIII) (Pause *et al.*, (1997) Proc. Natl. Acad. Sci. USA 94, 2156-2161). Human CUL-2 is a close homologue of CUL-1 while Elongin C/SIII shares substantial homology with SKP1 (Pause *et al.*, (1997) Proc. Natl. Acad. Sci. USA 94, 2156-2161; Kipreos *et al.*, (1996) Cell 85, 829-839). The formation of the VHL/CUL-2-Elongin C complex (Duan *et al.*, (1995) Science 269, 1402-1406; Kibel *et al.*, (1995) Science 269, 1444-1446), with additional components such as Elongin B (also called SIIIB, a ubiquitin-like protein) and Rbx1 (Kamura *et al.*, (1999) Science 284, 657-661; Duan *et al.*, (1995) Science 269, 1402-1406; Kibel *et al.*, (1995) Science 269, 1444-1446), has been suggested to contain an SCF-like ubiquitin ligase activity (Pause *et al.*, (1997) Proc. Natl. Acad. Sci. USA 94, 2156-2161). Immuno-purified SCF complexes from both yeast (Seol *et al.*, (1999) Genes Dev. 13, 1614-1626) and human cells can ubiquitinate proteins associated with SCF complexes if they are co-incubated with ubiquitin, ATP, CDC34 E2 conjugating enzyme and E1 (Figure 14). Similar ubiquitination activity has been found to associate with the purified VHL/CUL-2/Elongin C/Elongin B complex (Lisztwan *et al.*, (1999) Genes Dev. 13, 1822-1833). Since it was determined that VHL shares certain homology with the SCM of SKP2 in the  $\alpha$ -domain, it is likely VHL/CUL-2/Elongin C/Elongin B is an SCF-like E3 ubiquitin ligase that uses VHL as a substrate targeting subunit. Under such circumstances, the protein-knockout technique proposed for SKP2 or other F-box proteins can also be applied to the use of VHL. Thus it is expected that if one fuses a protein interaction domain with VHL, the VHL fusion protein should act to ubiquitinate the target protein through the interaction between the protein interaction domain and the target.

CUL-1 and CUL-2 belong to the cullin family (Kipreos *et al.*, (1996) Cell 85, 829-839), which so far contains several additional members such as CUL-3 (Singer *et al.*, (1999) Genes Dev. 13, 2375-2387; Michel & Xiong, (1998) Cell Growth Differ. 9, 435-449), CUL-4A and 4B (Kipreos *et al.*, (1996) Cell 85, 829-839; Chen *et al.*, (1998) Cancer Res. 58, 3677-3683), vasopressin-activated calcium-mobilizing receptor-1 (Stankovic *et al.*, (1997) Genomics 40, 267-276), and anaphase-promoting complex 2 (APC2) (Stankovic *et al.*, (1997) Genomics 40, 267-276). Based on the homology between CUL-1 and other members of cullin family, it is expected that these cullin family members should act as ubiquitin E3 ligases. In addition, if similar fusion proteins for the substrate-targeting components of these cullin family members are constructed, it is possible to alter the substrate specificity of these ubiquitin E3 ligases in the same design as proposed for that of SCF complexes.

#### Example 11 - F-box antagonist peptides block the SKP1/F-box protein interaction

The F-box region is a peptide motif composed of 40-50 amino acids that is present in a variety of otherwise unrelated proteins (Winston *et al.*, (1999) Curr. Biol. 9, 1180-1182; Cenciarelli *et al.*, (1999) Curr. Biol. 9, 1177-1179). The F-box region is required for the SKP1 interaction for the assembly of the SCF complex (SKP1, CUL-1, F-box proteins) (Zhang *et al.*, (1995) Cell 82, 915-925; Bai *et al.*, (1996) Cell 86, 263-274). Since F-box proteins regulate many important proteins such as  $\beta$ -catenin, I $\kappa$ B, p27, cyclin E that are involved in tumorigenesis, signal transduction, cell cycle regulation, and development (Maniatis, (1999) Genes Dev. 13, 505-510; Koepp *et al.*, (1999) Cell 97, 431-434; Sidow *et al.*, (1999) Nat. Genet. 23, 104-107; Kawakami *et al.*, (2000) Curr. Biol. 10, 463-466), it is anticipated that modulation of the various SCF complexes would provide a means to control and alter the biological consequences that involve the SCF activity. One way to interfere the SCF activity to alter the developmental, cell cycle, tumorigenic, or signaling pathways is to use the peptides or peptide analogues derived from the F-box region and use them as an antagonist peptide for SCF activities.

The method can be used for targeted protein knockout for genetic and biochemical analysis in cells and animals. It will help to elucidate the normal functions of a target protein in cells and animal or in human by creating deficient mutants of targeted protein.

It can also be used to correct the diseases by altering the level of the disease protein or its antagonists. It can be used for testing the function and regulation of the targeted protein in diseases, drug sensitivity, development, cell growth and differentiation, programmed cell death, behavior, gene expression patterns, and learning and memory.

- 5           The method can also be used for detecting protein-protein or peptide-protein interaction by fusing SKP2 or F-box proteins with a protein or peptide that bind to a target protein. Ubiquitination of the target protein can be used as the means of detection.

#### Example 12 - SKP2-Like Proteins

- 10           SKP2-like proteins are proteins that contain a SKP1 interacting domain that is homologous to the SKP2 sequence LPDELLLGIFSCCLPELLKVSGVCKRWYRL ASDESLWQTLDL (SEQ ID NO: 2) (amino acids 112-154) (Zhang *et al.*, (1995) Cell 82, 915-925; Bai *et al.*, (1996) Cell 86, 263-274; Patton *et al.*, (1998) Trends Genet. 14, 236-243; Skowyra *et al.*, (1997) Cell 91, 209-219; Yu *et al.*, (1998) Proc Natl Acad Sci USA.  
15           95, 11324-11329; Winston *et al.*, (1999) Genes Dev. 13, 270-283; Winston *et al.*, (1999) Curr. Biol. 9, 1180-1182; Cenciarelli *et al.*, (1999) Curr. Biol. 9, 1177-1179). The SKP1 interacting domain is the region on the SKP2 protein that interacts with the SKP1 protein. This region is also called the F-box for SKP1 binding (Bai *et al.*, (1996) Cell 86, 263-274).

- The SKP1 interacting domain is present in a variety of proteins from yeast to human,  
20           including: (1) Xenopus b-TrCP which has the sequence LPARGLDHIAENILSYLDAKSL CSAELVCKEWYRV TSDGMLWKKL (SEQ ID NO: 3) (amino acids 135-157); (2) human b-TrCP (amino acids 148-192), which is identical to SEQ ID NO: 3 (Bai *et al.*, (1996) Cell 86, 263-274; Winston *et al.*, (1999) Genes Dev. 13, 270-283; Spevak *et al.*, (1993) Mol. Cell. Biol. 13, 4953-4966); and (3) some yeast proteins such as CDC4 and  
25           GRR1 (Bai *et al.*, (1996) Cell 86, 263-274; Skowyra *et al.*, (1997) Cell 91, 209-219).

- These proteins can replace SKP2 to form a complex with SKP1 and CUL-1 or their yeast homologues SKP1 or CDC53. Like SKP2, they bind to phosphorylated proteins and target them for ubiquitination and degradation. More than ten human SKP2-like proteins have been identified and obtained through ESTdatabase (Figure 12) (Winston *et al.*,  
30           (1999) Curr. Biol. 9, 1180-1182; Cenciarelli *et al.*, (1999) Curr. Biol. 9, 1177-1179. See also, for example, SEQ ID NO: 26-61.

Although the present invention has been described in detail with reference to examples above, it is understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims. All cited patents and publications referred to in this application are

5 . herein incorporated by reference in their entirety.

We claim,

1. A method of altering the level of polypeptide in a cell comprising altering the amount of one or more of the proteins selected from the group consisting of SKP1, SKP2,  
5 SKP2-like protein and CUL-1.
2. The method of claim 1 wherein the polypeptide is phosphorylated.
3. The method of claim 1 wherein the SKP-2 like protein is selected from the  
10 group consisting of ZF1 (SEQ ID NO: 27), ZF3 (SEQ ID NO: 29), ZF4 (SEQ ID NO: 31), ZF5 (SEQ ID NO: 33), ZF6 (SEQ ID NO: 35), ZF7 (SEQ ID NO: 37), ZF8 (SEQ ID NO: 39), ZF9 (SEQ ID NO: 41), ZF11 (SEQ ID NO: 43), ZF13 (SEQ ID NO: 45), ZF16 (SEQ ID NO: 47), ZF18 (SEQ ID NO: 49), ZF19 (SEQ ID NO: 51), ZF20 (SEQ ID NO: 53), ZF23 (SEQ ID NO: 55), ZF24 (SEQ ID NO: 57), ZF25 (SEQ ID NO: 59) and ZF26 (SEQ  
15 ID NO: 61).
4. The method of claim 1 wherein the polypeptide is p27 (SEQ ID NO: 65).
5. The method of claim 1 wherein the polypeptide is selected from the group  
20 consisting of cyclin E (SEQ ID NO: 63), Max (SEQ ID NO: 9), Mad (SEQ ID NO: 11), c-Myc (SEQ ID NO: 13), MDM2 (SEQ ID NO: 15), p53 (SEQ ID NO: 17), Bax (SEQ ID NO: 19), Bad (SEQ ID NO: 21) and Bcl-2 (SEQ ID NO: 23).
6. The method of claim 1 wherein the level of polypeptide is increased by  
25 decreasing the amount of SKP2.
7. The method of claim 1 wherein the level of polypeptide is reduced by increasing the amount of SKP2.
- 30 8. A method of altering the level of SKP2 comprising altering the amount of p27 polypeptide which is available for binding with SKP2.

9. A method of modulating the activity of SKP2 comprising contacting SKP2 with a peptide comprising a SKP2 interaction domain which is available for binding with SKP2.

5

10. The method of claim 9 wherein the peptide is phosphorylated.

11. The method of claim 10 wherein the SKP2 interaction domain is derived from p27.

10

12. The method of claim 10 wherein the SKP2 interaction domain is derived from cyclin E.

13. The method of claim 9 wherein the peptide comprises any one of the amino acid sequences of SEQ ID NO: 1, 2, 3, 4, 5, or 6.

15

14. A method of treating a tumor in a mammal comprising altering the level of SKP protein in the cells of said tumor.

15. The method of claim 14 wherein the SKP protein is SKP2 or allelic variants thereof.

20

16. A method of detecting a tumor in a mammal wherein the level of SKP2 is used as a diagnostic indicator to determine the progression of said tumor.

25

17. A method of detecting a tumor in a mammal wherein the level of SKP2 is used as a prognostic indicator to determine the progression of said tumor.

18. A method of monitoring the treatment of a tumor in a mammal wherein the level of SKP2 is used as a diagnostic indicator to monitor the success of a said treatment.

30

19. A method of monitoring the treatment of a tumor in a mammal wherein the level of SKP2 is used as a prognostic indicator to monitor the success of a said treatment.

20. A method of testing an agent for the ability to modulate an interaction between SKP2 and a target protein wherein the method comprises:

- (a) fusing SKP2 with a target protein interaction domain to produce a SKP2 fusion protein;
- (b) contacting the agent, the SKP2 fusion protein and the target protein; and
- (c) determining whether the interaction of the SKP2 fusion protein with the target protein has been modulated by the agent.

21. A method of altering the level of a target protein in a cell comprising inserting a heterologous target protein interaction domain into SKP2 or a SKP2-like protein to produce a fusion protein, and contacting the fusion protein with the target protein.

15

22. The method of claim 21 wherein the SKP-2 like protein is selected from the group consisting of ZF1 (SEQ ID NO: 27), ZF3 (SEQ ID NO: 29), ZF4 (SEQ ID NO: 31), ZF5 (SEQ ID NO: 33), ZF6 (SEQ ID NO: 35), ZF7 (SEQ ID NO: 37), ZF8 (SEQ ID NO: 39), ZF9 (SEQ ID NO: 41), ZF11 (SEQ ID NO: 43), ZF13 (SEQ ID NO: 45), ZF16 (SEQ ID NO: 47), ZF18 (SEQ ID NO: 49), ZF19 (SEQ ID NO: 51), ZF20 (SEQ ID NO: 53), ZF23 (SEQ ID NO: 55), ZF24 (SEQ ID NO: 57), ZF25 (SEQ ID NO: 59) and ZF26 (SEQ ID NO: 61).

20

23. A method of altering the level of a target protein in a cell comprising expressing a cDNA coding for a SKP2 fusion protein comprising a SKP2 protein fused with a target protein interaction domain which is specific for the target protein.

25

24. A method of ubiquitinating a target protein in a cell comprising fusing a target protein interaction domain with SKP2, and permitting the SKP2 fusion protein to contact with the target protein.

30



25. The method of either claim 23 or 24 wherein the target protein is selected from the group consisting of p27 (SEQ ID NO: 65), cyclin E (SEQ ID NO: 63), Max (SEQ ID NO: 9), Mad (SEQ ID NO: 11), c-Myc (SEQ ID NO: 13), MDM2 (SEQ ID NO: 15), p53 (SEQ ID NO: 17), Bax (SEQ ID NO: 19), Bad (SEQ ID NO: 21) and Bcl-2 (SEQ ID NO: 23).

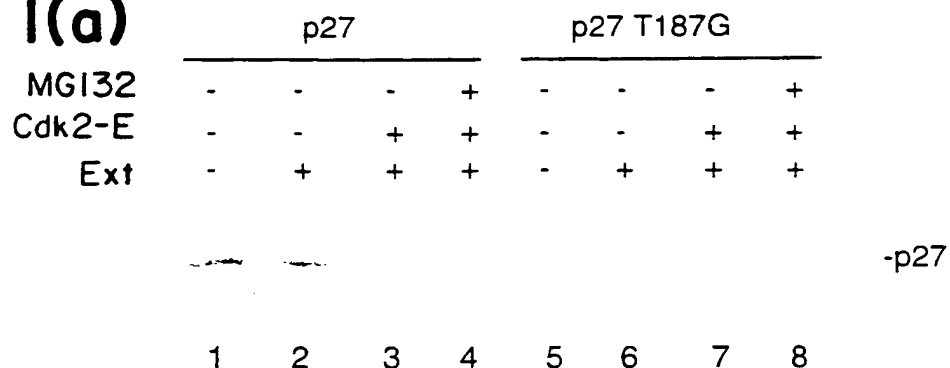
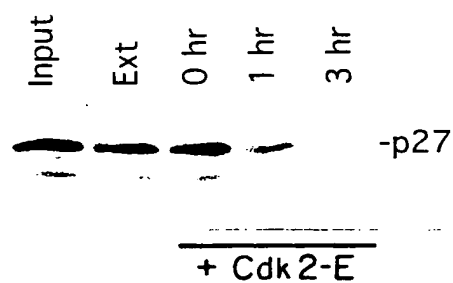
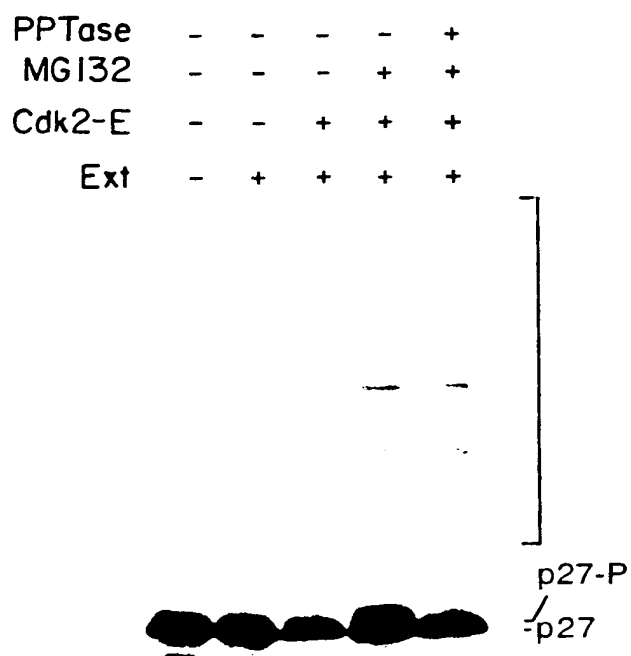
26. A method of modulating protein ubiquitination in a cell comprising altering the amount of SKP2 which is available to facilitate protein ubiquitination.

27. A fusion protein comprising a first protein comprising at least one SKP2 C-terminal motif (SCM) capable of interacting with SKP1 and forming a complex with CUL-1 and a second protein which is capable of interacting with a heterologous target protein.

28. The fusion protein of claim 27 wherein the fusion protein contains only one SCM capable of interacting with SKP1.

29. The fusion protein of claim 27 wherein the SCM is selected from any one of the following proteins selected from the group consisting of SKP2 (SEQ ID NO: 67), ZF1 (SEQ ID NO: 27), ZF3 (SEQ ID NO: 29), ZF4 (SEQ ID NO: 31), ZF5 (SEQ ID NO: 33), ZF6 (SEQ ID NO: 35), ZF7 (SEQ ID NO: 37), ZF8 (SEQ ID NO: 39), ZF9 (SEQ ID NO: 41), ZF11 (SEQ ID NO: 43), ZF13 (SEQ ID NO: 45), ZF16 (SEQ ID NO: 47), ZF18 (SEQ ID NO: 49), ZF19 (SEQ ID NO: 51), ZF20 (SEQ ID NO: 53), ZF23 (SEQ ID NO: 55), ZF24 (SEQ ID NO: 57), ZF25 (SEQ ID NO: 59) and ZF26 (SEQ ID NO: 61).

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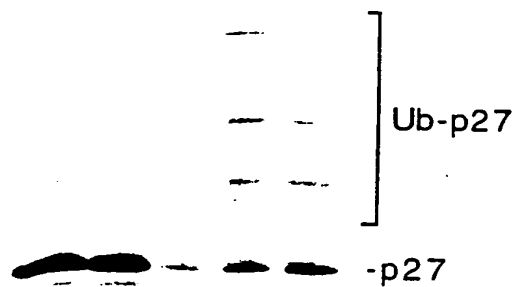
**FIG. 1(a)****FIG. 1(b)****FIG. 1(c)**

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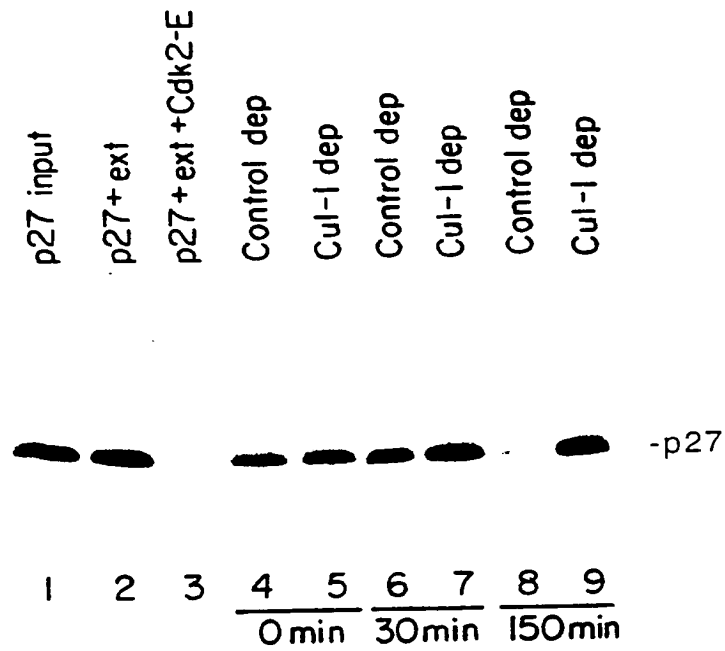
**FIG. 2**

MGI32	-	-	-	-	+
UbM+UbA	-	+	-	+	+
Cdk2-E	-	-	+	+	+
Extract	-	+	+	+	+

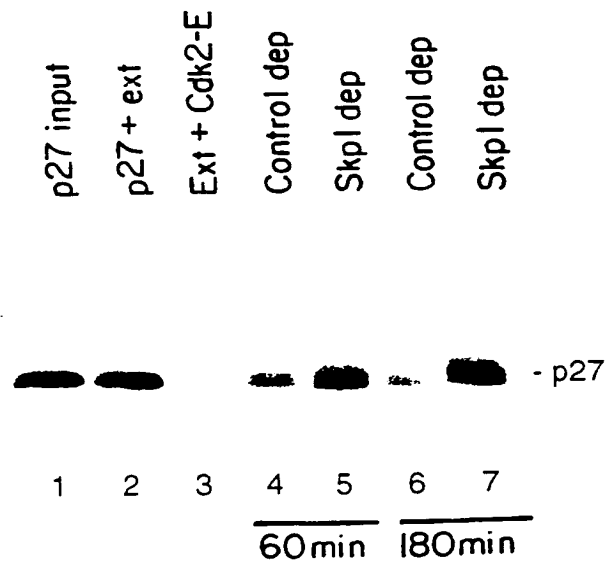


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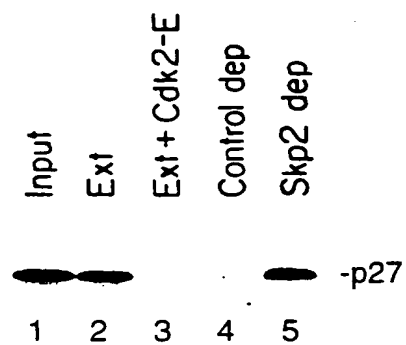
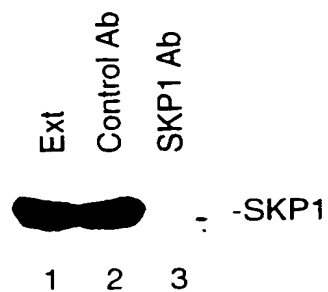
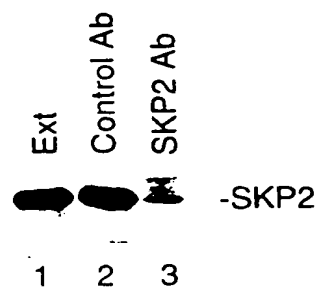
**FIG. 3(a)**



**FIG. 3(b)**



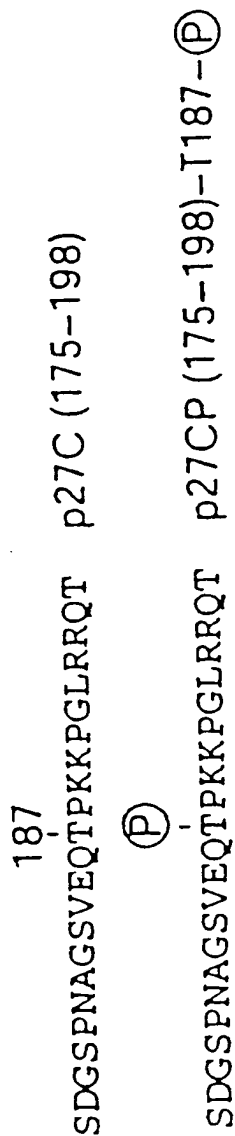
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**FIG. 3(c)****FIG. 3(d)**

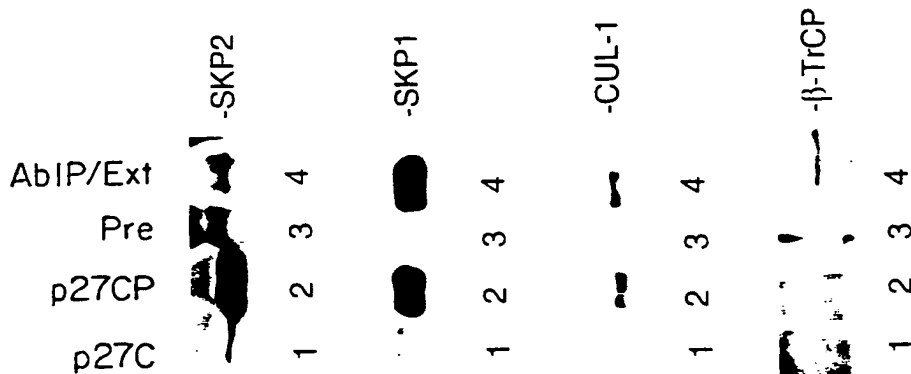
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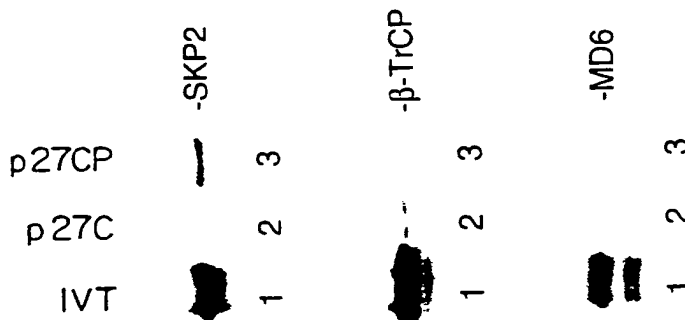
**FIG. 4(a)**



**FIG. 4(c)**



**FIG. 4(b)**



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FIG. 4(d)

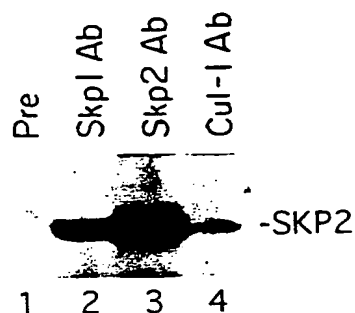
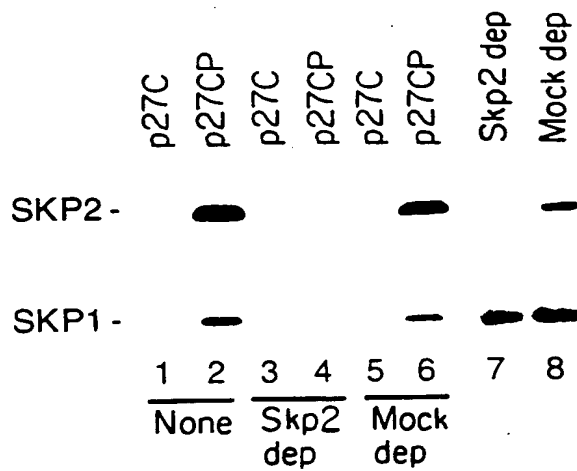


FIG. 4(e)



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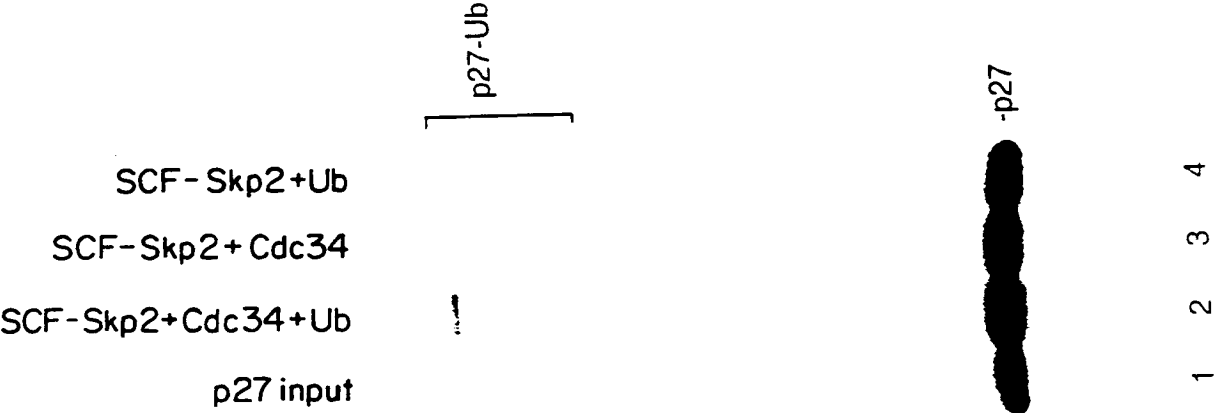
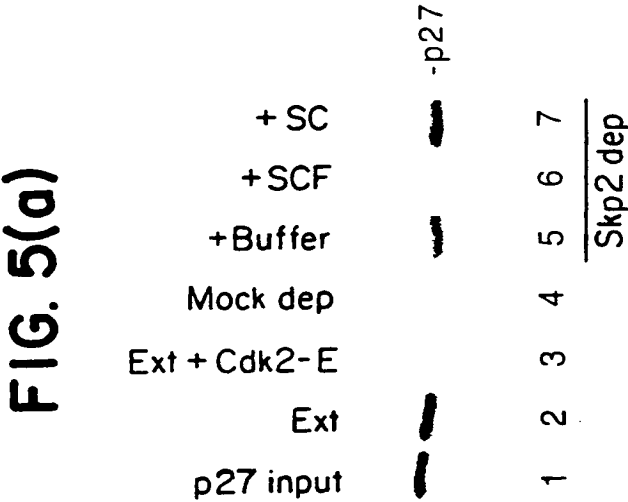


FIG. 5(b)



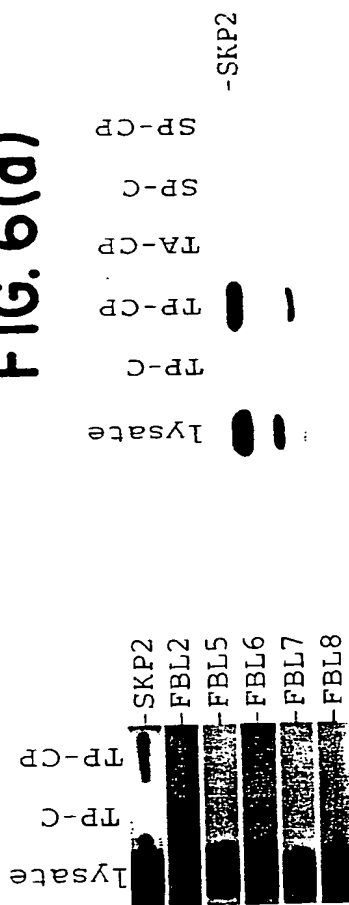


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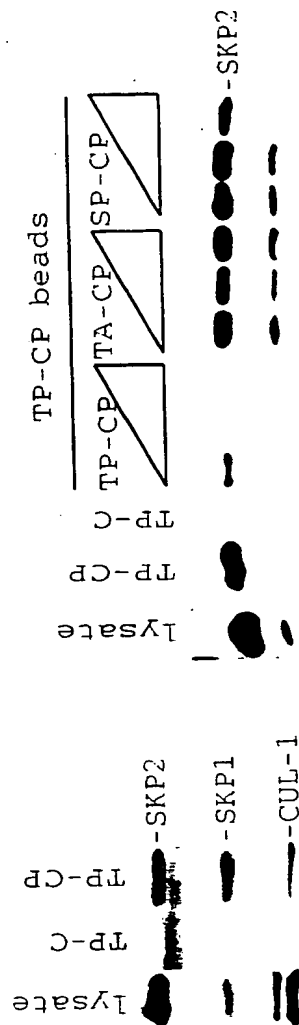
**FIG. 6(a)** Cyclin E peptides:

ASPLPSGLLTTPPQSGKKQSSGP<sup>Ⓟ</sup>EM TP-C (a.a. 371-394)  
 ASPLPSGLLTTPPQSGKKQSSGP<sup>Ⓟ</sup>EM TP-CP

**FIG. 6(d)**



**FIG. 6(b)**

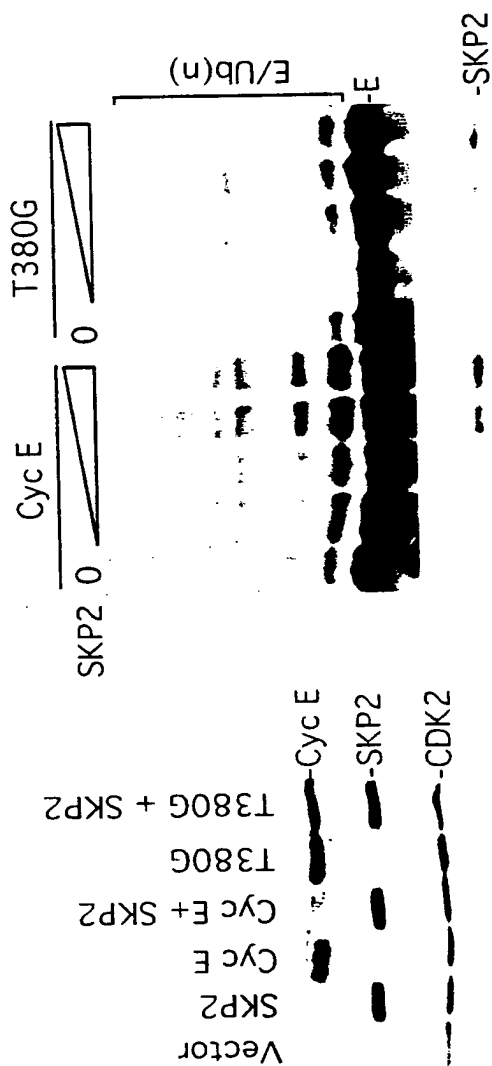


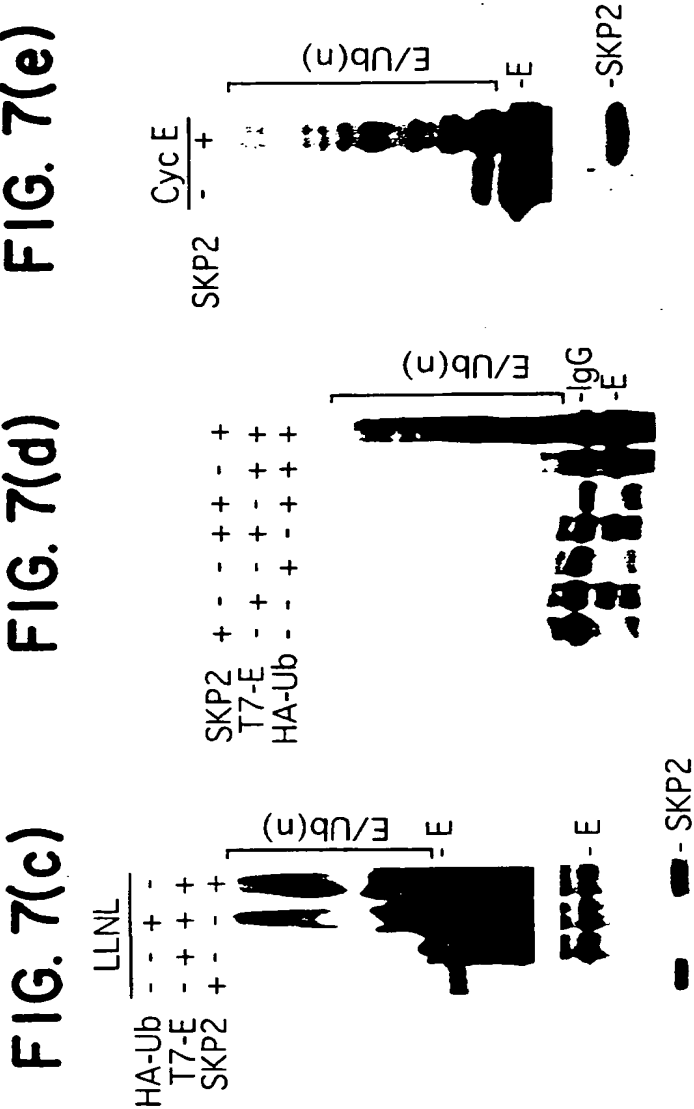
**FIG. 6(c)**

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FIG. 7(a)

FIG. 7(b)





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FIG. 8(a)

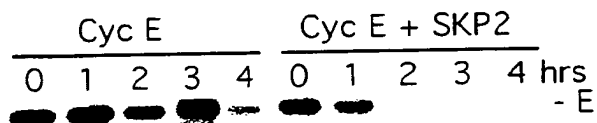


FIG. 8(b)

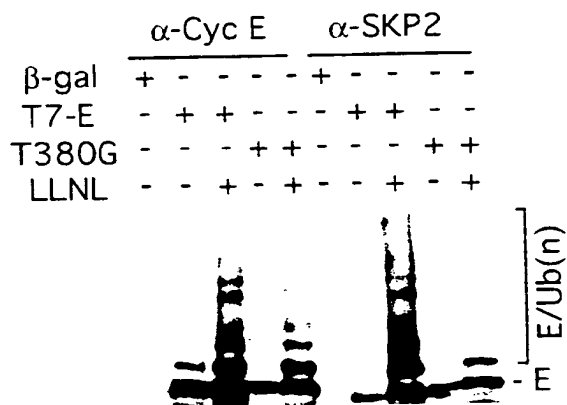
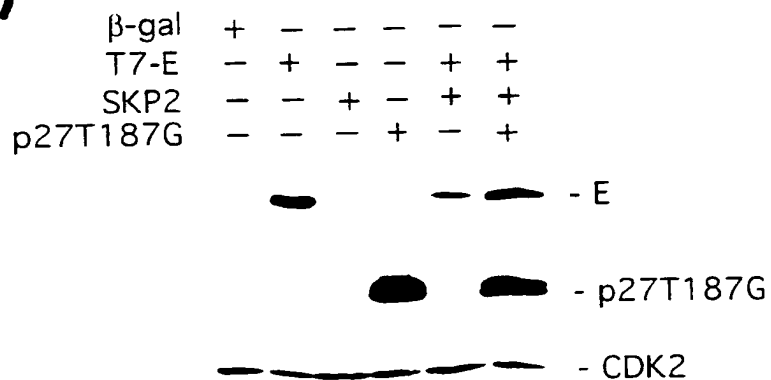
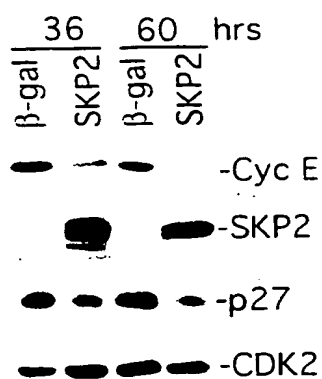
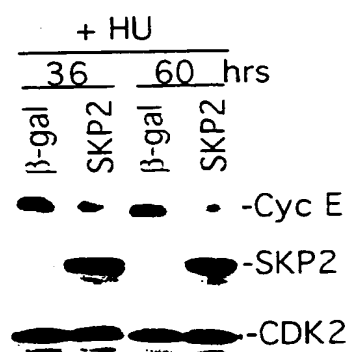
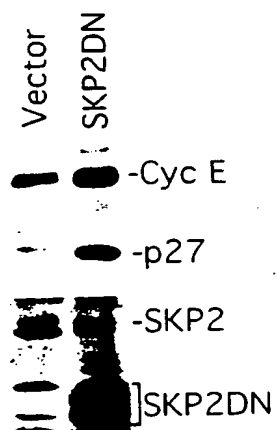
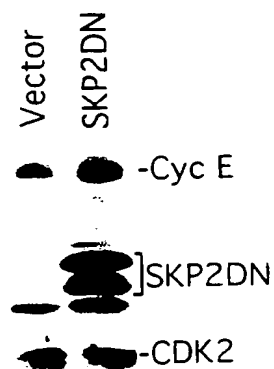


FIG. 8(c)



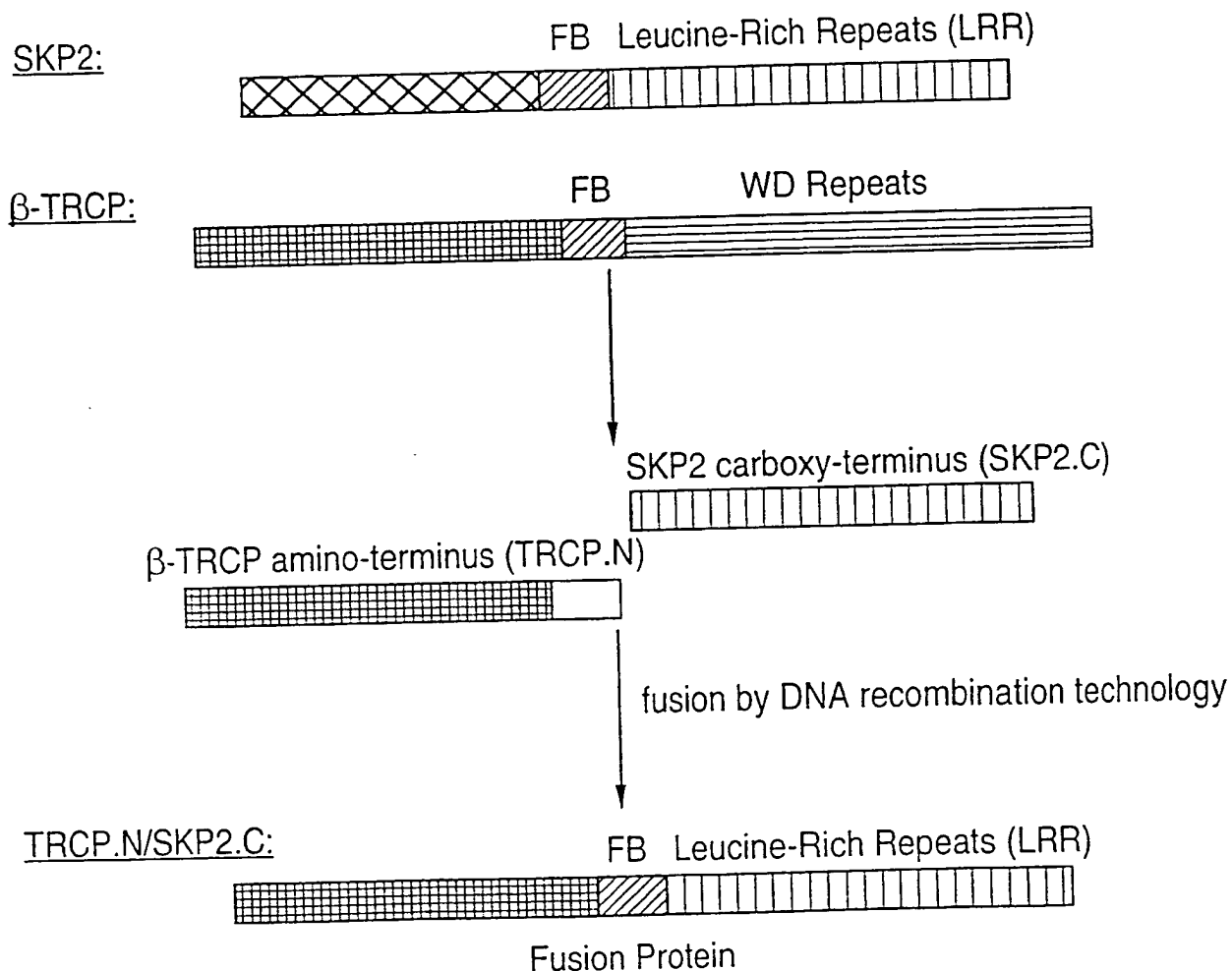
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**FIG. 9(a)****FIG. 9(b)****FIG. 9(c)****FIG. 9(d)**

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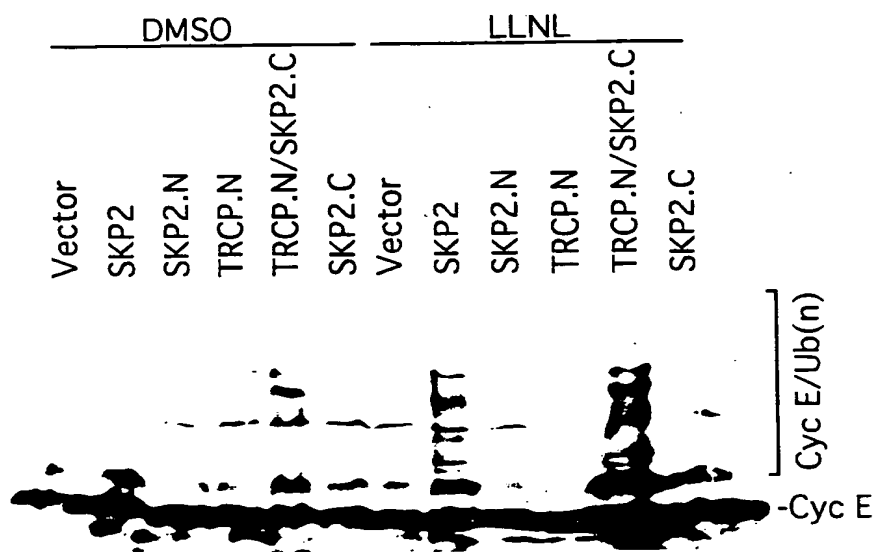
**FIG. 10**

Creating a hybrid fusion protein (TRCP.N/SKP2.C) consisting of the amino-terminal region of  $\beta$ -TRCP (a.a. 1-204) and the carboxy-terminal region of SKP2 (a.a. 169-435):



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**FIG. II**



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# **FIG. 12** Homologies between SKP2 and other F-box proteins in the F-box region:

	1	15	16	30	31	45	46	60	61	67	
1	SKP2	LPD----	ELLLGIFS	CLCLPE-----	LLKV	SGVCKRWY-RLASDE	SLWQTLDL-----				43
2	ZF25	LPD----	SVLLEIFS	YLPVRD-----	RIRI	SRVCHRWK-RLVDDR	WLWRHVDLT-----				44
3	ZF16	IPL----	EILVQIFG	LLVAADGMPFGLGRA	ARVCRRWQ-EAASQP	ALWHTVTTL-----					48
4	ZF24	LPD----	HSMVQIFS	FLPTNQ-----	LCRC	ARVCRRWY-NLAWDP	RLWRTIRLT-----				44
5	ZF8	LPI----	DVQLYILS	FLSPHD-----	LCQL	GSTNHYNW-ETVRDP	ILWRYFLLR-----				44
6	ZF20	LPI----	DVQLYILS	FLSPHD-----	LCQL	GSTNHYNW-ETVRDP	ILWRYFLLRD-----				45
7	ZF5	LPR----	VLSVYIFS	FLDPRS-----	LCRC	AQVSWYWK-SLAELD	QLWMLKCL-----				43
8	ZF7	LPY----	ELAINIFQ	YLDRKE-----	LGRC	AQVSKTWK-VIAEDE	VLWYRLCQQ-----				44
9	ZF1	LPK----	ELLRLRIFS	FLDIVT-----	LCRC	AQISKAWN-ILALDG	SNWQRID-----				42
10	ZF3	LPP----	EVMLSIFS	YLN PQE-----	LCRC	SQVSMKWS-QLTKTG	SLWKHLYPVHWARGD	WYSGPAT			57
11	ZF4	LPD----	EVVLKIFS	YLLEQD-----	LCRA	ACVCKRFS-ELANDP	ILWLGEVAHA-----				45
12	ZF9	LPP----	ELSFTILS	YLNATD-----	LCLA	SCVWQ----DLANDE	LLWQGLCK-----				40
13	ZF11	LPARGLDHIAENILS	YLDAKS-----	LCAA	ELVCKEWY-RVTSDG	MLWKKLIE-----					47
14	ZF13	LPS----	VPMMEILS	YLDAYS-----	LLQA	AQVNKNWN-ELASSD	VLWRKLC-----				42
15	ZF23	LPT----	DPLLLILS	FLDYRD-----	LINC	CYVSRRLS-QLSSHD	PLWRRHCKKYWLISE	EE-----			52
16	ZF19	LPLLP-DSL VYQIFL	SLGPAD-----	VLAA	GLVCRQWQ-AVSRDE	FLWKEQFYR-----					47
17	ZF18	LLQ----	DIILQVFK	YLPLLD-----	RAHA	SQVCRNWN-QVPHMP	DLWRCFEFE-----				44
18	ZF26	LPE----	VLLLLHMC	YLD MRA-----	LGRL	AQVYRWLW-HFTNCD	LLRRQIAWASLNSG-				49
19	ZF6	LPL----	ELSFYLLK	WLDPQT-----	LLTC	CLVSKQRNKNVISACT	EVWQTACKNLGWQID	DSV-----			54

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## FIG. 13

Homology between the  $\alpha$  domain of the von Hippel-Linda protein (VHL) and the SCM domain of SKP2:

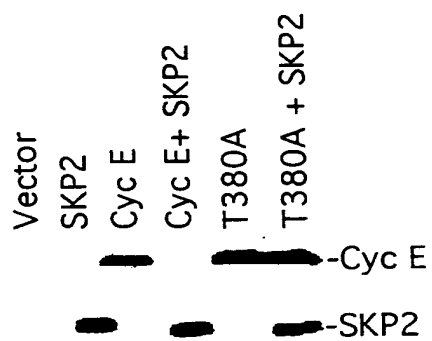
VHL: PIFANITLP - VYTLKERCLQVVRSLVKPEN - - - YRRLDIVRSLYEDLEDHPNVQ  
SKP2: PNLVHLDLSNSVMLKND CFQEFSQLNYLQHLSLRCYDI I PETLLELGEI PTLK

Homology between the SCM domain of  $\beta$ -TRCP, SKP2, and CDC4.Ca:

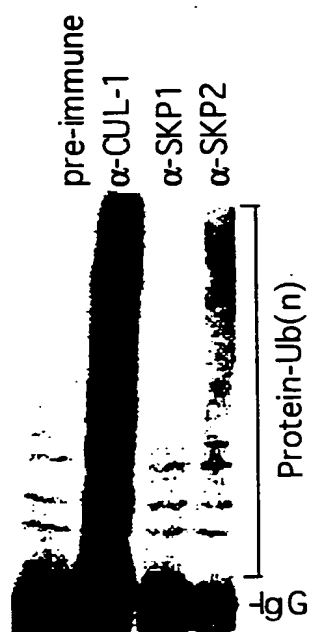
$\beta$ -TRCP: LRVLEGHEELVRCIRFDNKRI VSGAYDGKIKVWDL  
SKP2: QLNYLQHLSLRCYDI I PETLLELGEI PTLKTLQV  
CDC4.Ca: THVFKGHNSTVRCLDIVEYKNIKIYI VTGSRDNTLH

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**FIG. 14**

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**FIG. 15**

## SEQUENCE LISTING

<110> Zhang, Hui  
Tsvetkov, Lyuben  
Kondo, Takeshi

<120> Modulation of Protein Levels Using the SCF Complex

<130> 44574-5047-WO

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20 25

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<223> Amino acids 112-154 of SKP2-like protein

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1 5 10 15

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1 5 10 15  
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20 25 30  
Tyr Arg Val Thr Ser Asp Gly Met Leu Trp Lys Lys Leu  
35 40 45

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Gln Ser Ser Gly Pro Glu Met Ala  
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<223> Description of Artificial Sequence: Cyclin E  
peptide

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1 5 10 15  
Lys Lys Gln Ser Ser Gly Pro Glu Met  
20 25

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<223> Description of Artificial Sequence: Cyclin E  
mutant peptide

&lt;400&gt; 6

Cys	Ala	Ser	Pro	Leu	Pro	Ser	Gly	Leu	Leu	Thr	Ala	Pro	Gln	Ser	Gly
1				5				10					15		

Lys	Lys	Gln	Ser	Ser	Gly	Pro	Glu	Met
		20					25	

&lt;210&gt; 7

&lt;211&gt; 25

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Cyclin E  
mutant peptide

&lt;400&gt; 7

Cys	Ala	Ser	Pro	Leu	Pro	Ser	Gly	Leu	Leu	Ser	Pro	Pro	Gln	Ser	Gly
1				5				10					15		

Lys	Lys	Gln	Ser	Ser	Gly	Pro	Glu	Met
		20					25	

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&lt;211&gt; 623

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

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&lt;221&gt; CDS

&lt;222&gt; (22)..(330)

&lt;223&gt; Human Max

&lt;400&gt; 8

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			1				5									
agc	gac	gaa	gag	caa	ccg	agg	ttt	caa	tct	gcg	gct	gac	aaa	cgg	gct	99
Ser	Asp	Glu	Glu	Gln	Pro	Arg	Phe	Gln	Ser	Ala	Ala	Asp	Lys	Arg	Ala	
			15					20					25			
cat	cat	aat	gca	ctg	gaa	cga	aaa	cgt	agg	gac	cac	atc	aaa	gac	agc	147
His	His	Asn	Ala	Leu	Glu	Arg	Lys	Arg	Arg	Asp	His	Ile	Lys	Asp	Ser	
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ttt	cac	agt	ttg	cgg	gac	tca	gtc	cca	tca	ctc	caa	gga	gag	aag	gca	195
Phe	His	Ser	Leu	Arg	Asp	Ser	Val	Pro	Ser	Leu	Gln	Gly	Glu	Lys	Ala	
			45				50					55				
tcc	cgg	gcc	caa	atc	cta	gac	aaa	gcc	aca	gaa	tat	atc	cag	tat	atg	243
Ser	Arg	Ala	Gln	Ile	Leu	Asp	Lys	Ala	Thr	Glu	Tyr	Ile	Gln	Tyr	Met	

}

60 65 70

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 Arg Arg Lys Asn His Thr His Gln Gln Asp Ile Asp Asp Leu Lys Arg  
 75 80 85 90

cag aat gct ctt ctg gag cag caa ggg gaa agc gag agc tgatcaagtt 340  
 Gln Asn Ala Leu Leu Glu Gln Gln Gly Glu Ser Glu Ser  
 95 100

ctttgttcct ggggaattca cttctcttcc ttctctcatgg aagatgcaag taaaaggaaa 400

tgcaagtaac cacctggtcc gtgcactgga gaaggcgagg tcaagtgccc aactgcagac 460

caactacccc tcctcagaca acagcctcta caccaacgcc aagggcagca ccatttctgc 520

cttcgatggg ggctcggact ccagctcgga gtctgagcct gaagagcccc aaagcaggaa 580

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Arg Lys Arg Arg Asp His Ile Lys Asp Ser Phe His Ser Leu Arg Asp  
 35 40 45

Ser Val Pro Ser Leu Gln Gly Glu Lys Ala Ser Arg Ala Gln Ile Leu  
 50 55 60

Asp Lys Ala Thr Glu Tyr Ile Gln Tyr Met Arg Arg Lys Asn His Thr  
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Gln Gln Gly Glu Ser Glu Ser  
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tcccgtggct ccggcccccg gtgcaga atg gcg gcg gcg gtt cgg atg aac atc 174  
Met Ala Ala Ala Val Arg Met Asn Ile  
1 5

cag atg ctg ctg gag gcg gcc gac tat ctg gag cgg cgg gag aga gaa 222  
Gln Met Leu Leu Glu Ala Ala Asp Tyr Leu Glu Arg Arg Glu Arg Glu  
10 15 20 25

gct gaa cat ggt tat gcc tcc atg tta cca tac aat aac aag gac aga 270  
Ala Glu His Gly Tyr Ala Ser Met Leu Pro Tyr Asn Asn Lys Asp Arg  
30 35 40

gat gcc tta aaa cgg agg aac aaa tcc aaa aag aat aac agc agt agc 318  
Asp Ala Leu Lys Arg Arg Asn Lys Ser Lys Lys Asn Asn Ser Ser Ser  
45 50 55

aga tca act cac aat gaa atg gag aag aat aga cgg gct cat ctt cgc 366  
Arg Ser Thr His Asn Glu Met Glu Lys Asn Arg Arg Ala His Leu Arg  
60 65 70

ttg tgc ctg gag aag ttg aag ggg ctg gtg cca ctg gga ccc gaa tca 414  
Leu Cys Leu Glu Lys Leu Lys Gly Leu Val Pro Leu Gly Pro Glu Ser  
75 80 85

agt cga cac act acg ttg agt tta tta aca aaa gcc aaa ttg cac ata 462  
Ser Arg His Thr Thr Leu Ser Leu Leu Thr Lys Ala Lys Leu His Ile  
90 95 100 105

aag aaa ctt gaa gat tgt gac aga aaa gcc gtt cac caa atc gac cag 510  
Lys Lys Leu Glu Asp Cys Asp Arg Lys Ala Val His Gln Ile Asp Gln  
110 115 120

ctt cag cga gag cag cga cac ctg aag agg cag ctg gag aag ctg ggc 558  
Leu Gln Arg Glu Gln Arg His Leu Lys Arg Gln Leu Glu Lys Leu Gly  
125 130 135

att gag agg atc cgg atg gac agc atc ggc tcc acc gtc tcc tcg gag 606  
Ile Glu Arg Ile Arg Met Asp Ser Ile Gly Ser Thr Val Ser Ser Glu  
140 145 150

cgc tcc gac tcc gac agg gaa gaa atc gac gtt gac gtg gag agc acg 654  
Arg Ser Asp Ser Asp Arg Glu Glu Ile Asp Val Asp Val Glu Ser Thr  
155 160 165

gac tat ctc aca ggt gat ctg gac tgg agc agc agc agt gtg agc gac 702  
Asp Tyr Leu Thr Gly Asp Leu Asp Trp Ser Ser Ser Ser Val Ser Asp  
170 175 180 185

tct gac gag cgg ggc agc atg cag agc ctc ggc agt gat gag ggc tat 750  
Ser Asp Glu Arg Gly Ser Met Gln Ser Leu Gly Ser Asp Glu Gly Tyr  
190 195 200



tcc agc acc agc atc aag aga ata aag ctg cag gac agt cac aag gcg 798  
 Ser Ser Thr Ser Ile Lys Arg Ile Lys Leu Gln Asp Ser His Lys Ala  
                   205                                  210                                  215

tgt ctt ggt ctc taagagagtg ggcactgcgg ctgtctcctt gaagggtctc 850  
 Cys Leu Gly Leu  
                   220

cctgttggtt ctgattaggt aacgtattgg acctgcccac aactcccttg cacgtaaact 910  
 tcagtgtccc accttgacca aaatcagctt tgtaactgtt ttcaaggagg tgcttaggat 970  
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 <213> Homo sapiens

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                   20                                  25                                  30

Met Leu Pro Tyr Asn Asn Lys Asp Arg Asp Ala Leu Lys Arg Arg Asn  
                   35                                  40                                  45

Lys Ser Lys Lys Asn Asn Ser Ser Ser Arg Ser Thr His Asn Glu Met  
                   50                                  55                                  60

Glu Lys Asn Arg Arg Ala His Leu Arg Leu Cys Leu Glu Lys Leu Lys  
   65                                  70                                  75                                  80

Gly Leu Val Pro Leu Gly Pro Glu Ser Ser Arg His Thr Thr Leu Ser  
                                   85                                  90                                  95

Leu Leu Thr Lys Ala Lys Leu His Ile Lys Lys Leu Glu Asp Cys Asp  
                   100                                  105                                  110

Arg Lys Ala Val His Gln Ile Asp Gln Leu Gln Arg Glu Gln Arg His  
                   115                                  120                                  125

Leu Lys Arg Gln Leu Glu Lys Leu Gly Ile Glu Arg Ile Arg Met Asp  
                   130                                  135                                  140

Ser Ile Gly Ser Thr Val Ser Ser Glu Arg Ser Asp Ser Asp Arg Glu  
   145                                  150                                  155                                  160

Glu Ile Asp Val Asp Val Glu Ser Thr Asp Tyr Leu Thr Gly Asp Leu  
                                   165                                  170                                  175

Asp Trp Ser Ser Ser Ser Val Ser Asp Ser Asp Glu Arg Gly Ser Met  
                   180                                  185                                  190

Gln Ser Leu Gly Ser Asp Glu Gly Tyr Ser Ser Thr Ser Ile Lys Arg

195                      200                      205

Ile Lys Leu Gln Asp Ser His Lys Ala Cys Leu Gly Leu

210                      215                      220

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ataaaagccg gtttttcgggg ctttatctaa ctgcgtgtag taattccagc gagaggcaga 180

gggagcgagc gggcgggccgg ctaggggtgga agagccgggc gagcagagct gcgctgcggg 240

cgtcctggga agggagatcc ggagcgaata gggggcttcg cctctggccc agccctcccg 300

cttgatcccc caggccagcg gtccgcaacc cttgccgcat ccacgaaact ttgcccatag 360

cagcggggcg gcactttgca ctggaactta caacaccga gcaaggacgc gactctcccg 420

acgcgggggag gctattctgc ccatttgggg acacttcccc gccgctgcca ggacccgctt 480

ctctgaaagg ctctccttgc agctgcttag acgctggatt tttttcgggt agtggaaaac 540

cagcagcctc ccgcgacg atg ccc ctc aac gtt agc ttc acc aac agg aac 591

Met Pro Leu Asn Val Ser Phe Thr Asn Arg Asn

1                      5                      10

tat gac ctc gac tac gac tgc gtg cag ccg tat ttc tac tgc gac gag 639

Tyr Asp Leu Asp Tyr Asp Ser Val Gln Pro Tyr Phe Tyr Cys Asp Glu

15                      20                      25

gag gag aac ttc tac cag cag cag cag cag agc gag ctg cag ccc ccg 687

Glu Glu Asn Phe Tyr Gln Gln Gln Gln Gln Ser Glu Leu Gln Pro Pro

30                      35                      40

gcg ccc agc gag gat atc tgg aag aaa ttc gag ctg ctg ccc acc ccg 735

Ala Pro Ser Glu Asp Ile Trp Lys Lys Phe Glu Leu Leu Pro Thr Pro

45                      50                      55

ccc ctg tcc cct agc cgc cgc tcc ggg ctc tgc tgc ccc tcc tac gtt 783

Pro Leu Ser Pro Ser Arg Arg Ser Gly Leu Cys Ser Pro Ser Tyr Val

60                      65                      70                      75

gcg gtc aca ccc ttc tcc ctt cgg gga gac aac gac ggc ggt ggc ggg 831

Ala Val Thr Pro Phe Ser Leu Arg Gly Asp Asn Asp Gly Gly Gly Gly

80	85	90	
agc ttc tcc acg gcc gac cag ctg gag atg gtg acc gag ctg ctg gga			879
Ser Phe Ser Thr Ala Asp Gln Leu Glu Met Val Thr Glu Leu Leu Gly			
95	100	105	
gga gac atg gtg aac cag agt ttc atc tgc gac ccg gac gac gag acc			927
Gly Asp Met Val Asn Gln Ser Phe Ile Cys Asp Pro Asp Asp Glu Thr			
110	115	120	
ttc atc aaa aac atc atc atc cag gac tgt atg tgg agc ggc ttc tcg			975
Phe Ile Lys Asn Ile Ile Ile Gln Asp Cys Met Trp Ser Gly Phe Ser			
125	130	135	
gcc gcc gcc aag ctc gtc tca gag aag ctg gcc tcc tac cag gct gcg			1023
Ala Ala Ala Lys Leu Val Ser Glu Lys Leu Ala Ser Tyr Gln Ala Ala			
140	145	150	155
cgc aaa gac agc ggc agc ccg aac ccc gcc cgc ggc cac agc gtc tgc			1071
Arg Lys Asp Ser Gly Ser Pro Asn Pro Ala Arg Gly His Ser Val Cys			
160	165	170	
tcc acc tcc agc ttg tac ctg cag gat ctg agc gcc gcc gcc tca gag			1119
Ser Thr Ser Ser Leu Tyr Leu Gln Asp Leu Ser Ala Ala Ala Ser Glu			
175	180	185	
tgc atc gac ccc tcg gtg gtc ttc ccc tac cct ctc aac gac agc agc			1167
Cys Ile Asp Pro Ser Val Val Phe Pro Tyr Pro Leu Asn Asp Ser Ser			
190	195	200	
tcg ccc aag tcc tgc gcc tcg caa gac tcc agc gcc ttc tct ccg tcc			1215
Ser Pro Lys Ser Cys Ala Ser Gln Asp Ser Ser Ala Phe Ser Pro Ser			
205	210	215	
tcg gat tct ctg ctc tcc tcg acg gag tcc tcc ccg cag ggc agc ccc			1263
Ser Asp Ser Leu Leu Ser Ser Thr Glu Ser Ser Pro Gln Gly Ser Pro			
220	225	230	235
gag ccc ctg gtg ctc cat gag gag aca ccg ccc acc acc agc agc gac			1311
Glu Pro Leu Val Leu His Glu Glu Thr Pro Pro Thr Thr Ser Ser Asp			
240	245	250	
tct gag gag gaa caa gaa gat gag gaa gaa atc gat gtt gtt tct gtg			1359
Ser Glu Glu Glu Gln Glu Asp Glu Glu Glu Ile Asp Val Val Ser Val			
255	260	265	
gaa aag agg cag gct cct ggc aaa agg tca gag tct gga tca cct tct			1407
Glu Lys Arg Gln Ala Pro Gly Lys Arg Ser Glu Ser Gly Ser Pro Ser			
270	275	280	
gct gga ggc cac agc aaa cct cct cac agc cca ctg gtc ctc aag agg			1455
Ala Gly Gly His Ser Lys Pro Pro His Ser Pro Leu Val Leu Lys Arg			
285	290	295	
tgc cac gtc tcc aca cat cag cac aac tac gca gcg cct ccc tcc act			1503
Cys His Val Ser Thr His Gln His Asn Tyr Ala Ala Pro Pro Ser Thr			
300	305	310	315

cgg aag gac tat cct gct gcc aag agg gtc aag ttg gac agt gtc aga 1551  
 Arg Lys Asp Tyr Pro Ala Ala Lys Arg Val Lys Leu Asp Ser Val Arg  
 320 325 330

gtc ctg aga cag atc agc aac aac cga aaa tgc acc agc ccc agg tcc 1599  
 Val Leu Arg Gln Ile Ser Asn Asn Arg Lys Cys Thr Ser Pro Arg Ser  
 335 340 345

tcg gac acc gag gag aat gtc aag agg cga aca cac aac gtc ttg gag 1647  
 Ser Asp Thr Glu Glu Asn Val Lys Arg Arg Thr His Asn Val Leu Glu  
 350 355 360

cgc cag agg agg aac gag cta aaa cgg agc ttt ttt gcc ctg cgt gac 1695  
 Arg Gln Arg Arg Asn Glu Leu Lys Arg Ser Phe Phe Ala Leu Arg Asp  
 365 370 375

cag atc ccg gag ttg gaa aac aat gaa aag gcc ccc aag gta gtt atc 1743  
 Gln Ile Pro Glu Leu Glu Asn Asn Glu Lys Ala Pro Lys Val Val Ile  
 380 385 390 395

ctt aaa aaa gcc aca gca tac atc ctg tcc gtc caa gca gag gag caa 1791  
 Leu Lys Lys Ala Thr Ala Tyr Ile Leu Ser Val Gln Ala Glu Glu Gln  
 400 405 410

aag ctc att tct gaa gag gac ttg ttg cgg aaa cga cga gaa cag ttg 1839  
 Lys Leu Ile Ser Glu Glu Asp Leu Leu Arg Lys Arg Arg Glu Gln Leu  
 415 420 425

aaa cac aaa ctt gaa cag cta cgg aac tct tgt gcg taaggaaaag 1885  
 Lys His Lys Leu Glu Gln Leu Arg Asn Ser Cys Ala  
 430 435

taaggaaaac gattccttct aacagaaatg tcctgagcaa tcacctatga acttgtttca 1945  
 aatgcatgat caaatgcaac ctcaaacct tggctgagtc ttgagactga aagatttagc 2005  
 cataatgtaa actgcctcaa attggacttt gggcataaaa gaactttttt atgcttacca 2065  
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 <212> PRT  
 <213> Homo sapiens

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 Gln Gln Gln Gln Gln Ser Glu Leu Gln Pro Pro Ala Pro Ser Glu Asp  
 35 40 45  
 Ile Trp Lys Lys Phe Glu Leu Leu Pro Thr Pro Pro Leu Ser Pro Ser  
 50 55 60

Arg Arg Ser Gly Leu Cys Ser Pro Ser Tyr Val Ala Val Thr Pro Phe  
 65 70 75 80  
 Ser Leu Arg Gly Asp Asn Asp Gly Gly Gly Gly Ser Phe Ser Thr Ala  
 85 90 95  
 Asp Gln Leu Glu Met Val Thr Glu Leu Leu Gly Gly Asp Met Val Asn  
 100 105 110  
 Gln Ser Phe Ile Cys Asp Pro Asp Asp Glu Thr Phe Ile Lys Asn Ile  
 115 120 125  
 Ile Ile Gln Asp Cys Met Trp Ser Gly Phe Ser Ala Ala Ala Lys Leu  
 130 135 140  
 Val Ser Glu Lys Leu Ala Ser Tyr Gln Ala Ala Arg Lys Asp Ser Gly  
 145 150 155 160  
 Ser Pro Asn Pro Ala Arg Gly His Ser Val Cys Ser Thr Ser Ser Leu  
 165 170 175  
 Tyr Leu Gln Asp Leu Ser Ala Ala Ala Ser Glu Cys Ile Asp Pro Ser  
 180 185 190  
 Val Val Phe Pro Tyr Pro Leu Asn Asp Ser Ser Ser Pro Lys Ser Cys  
 195 200 205  
 Ala Ser Gln Asp Ser Ser Ala Phe Ser Pro Ser Ser Asp Ser Leu Leu  
 210 215 220  
 Ser Ser Thr Glu Ser Ser Pro Gln Gly Ser Pro Glu Pro Leu Val Leu  
 225 230 235 240  
 His Glu Glu Thr Pro Pro Thr Thr Ser Ser Asp Ser Glu Glu Glu Gln  
 245 250 255  
 Glu Asp Glu Glu Glu Ile Asp Val Val Ser Val Glu Lys Arg Gln Ala  
 260 265 270  
 Pro Gly Lys Arg Ser Glu Ser Gly Ser Pro Ser Ala Gly Gly His Ser  
 275 280 285  
 Lys Pro Pro His Ser Pro Leu Val Leu Lys Arg Cys His Val Ser Thr  
 290 295 300  
 His Gln His Asn Tyr Ala Ala Pro Pro Ser Thr Arg Lys Asp Tyr Pro  
 305 310 315 320  
 Ala Ala Lys Arg Val Lys Leu Asp Ser Val Arg Val Leu Arg Gln Ile  
 325 330 335  
 Ser Asn Asn Arg Lys Cys Thr Ser Pro Arg Ser Ser Asp Thr Glu Glu  
 340 345 350  
 Asn Val Lys Arg Arg Thr His Asn Val Leu Glu Arg Gln Arg Arg Asn  
 355 360 365  
 Glu Leu Lys Arg Ser Phe Phe Ala Leu Arg Asp Gln Ile Pro Glu Leu

370                      375                      380  
 Glu Asn Asn Glu Lys Ala Pro Lys Val Val Ile Leu Lys Lys Ala Thr  
 385                      390                      395                      400  
 Ala Tyr Ile Leu Ser Val Gln Ala Glu Glu Gln Lys Leu Ile Ser Glu  
                     405                      410                      415  
 Glu Asp Leu Leu Arg Lys Arg Arg Glu Gln Leu Lys His Lys Leu Glu  
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 Gln Leu Arg Asn Ser Cys Ala  
                     435

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 <222> (1)..(1473)  
 <223> Human MDM2

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   1                      5                      10                      15  
 tca cag att cca gct tcg gaa caa gag acc ctg gtt aga cca aag cca 96  
 Ser Gln Ile Pro Ala Ser Glu Gln Glu Thr Leu Val Arg Pro Lys Pro  
                     20                      25                      30  
 ttg ctt ttg aag tta tta aag tct gtt ggt gca caa aaa gac act tat 144  
 Leu Leu Leu Lys Leu Leu Lys Ser Val Gly Ala Gln Lys Asp Thr Tyr  
                     35                      40                      45  
 act atg aaa gag gtt ctt ttt tat ctt ggc cag tat att atg act aaa 192  
 Thr Met Lys Glu Val Leu Phe Tyr Leu Gly Gln Tyr Ile Met Thr Lys  
                     50                      55                      60  
 cga tta tat gat gag aag caa caa cat att gta tat tgt tca aat gat 240  
 Arg Leu Tyr Asp Glu Lys Gln Gln His Ile Val Tyr Cys Ser Asn Asp  
   65                      70                      75                      80  
 ctt cta gga gat ttg ttt ggc gtg cca agc ttc tct gtg aaa gag cac 288  
 Leu Leu Gly Asp Leu Phe Gly Val Pro Ser Phe Ser Val Lys Glu His  
                     85                      90                      95  
 agg aaa ata tat acc atg atc tac agg aac ttg gta gta gtc aat cag 336  
 Arg Lys Ile Tyr Thr Met Ile Tyr Arg Asn Leu Val Val Val Asn Gln  
                     100                      105                      110  
 cag gaa tca tcg gac tca ggt aca tct gtg agt gag aac agg tgt cac 384  
 Gln Glu Ser Ser Asp Ser Gly Thr Ser Val Ser Glu Asn Arg Cys His  
                     115                      120                      125

ctt gaa ggt ggg agt gat caa aag gac ctt gta caa gag ctt cag gaa	432
Leu Glu Gly Gly Ser Asp Gln Lys Asp Leu Val Gln Glu Leu Gln Glu	
130 135 140	
gag aaa cct tca tct tca cat ttg gtt tct aga cca tct acc tca tct	480
Glu Lys Pro Ser Ser Ser His Leu Val Ser Arg Pro Ser Thr Ser Ser	
145 150 155 160	
aga agg aga gca att agt gag aca gaa gaa aat tca gat gaa tta tct	528
Arg Arg Arg Ala Ile Ser Glu Thr Glu Glu Asn Ser Asp Glu Leu Ser	
165 170 175	
ggt gaa cga caa aga aaa cgc cac aaa tct gat agt att tcc ctt tcc	576
Gly Glu Arg Gln Arg Lys Arg His Lys Ser Asp Ser Ile Ser Leu Ser	
180 185 190	
ttt gat gaa agc ctg gct ctg tgt gta ata agg gag ata tgt tgt gaa	624
Phe Asp Glu Ser Leu Ala Leu Cys Val Ile Arg Glu Ile Cys Cys Glu	
195 200 205	
aga agc agt agc agt gaa tct aca ggg acg cca tcg aat ccg gat ctt	672
Arg Ser Ser Ser Ser Glu Ser Thr Gly Thr Pro Ser Asn Pro Asp Leu	
210 215 220	
gat gct ggt gta agt gaa cat tca ggt gat tgg ttg gat cag gat tca	720
Asp Ala Gly Val Ser Glu His Ser Gly Asp Trp Leu Asp Gln Asp Ser	
225 230 235 240	
gtt tca gat cag ttt agt gta gaa ttt gaa gtt gaa tct ctc gac tca	768
Val Ser Asp Gln Phe Ser Val Glu Phe Glu Val Glu Ser Leu Asp Ser	
245 250 255	
gaa gat tat agc ctt agt gaa gaa gga caa gaa ctc tca gat gaa gat	816
Glu Asp Tyr Ser Leu Ser Glu Glu Gly Gln Glu Leu Ser Asp Glu Asp	
260 265 270	
gat gag gta tat caa gtt act gtg tat cag gca ggg gag agt gat aca	864
Asp Glu Val Tyr Gln Val Thr Val Tyr Gln Ala Gly Glu Ser Asp Thr	
275 280 285	
gat tca ttt gaa gaa gat cct gaa att tcc tta gct gac tat tgg aaa	912
Asp Ser Phe Glu Glu Asp Pro Glu Ile Ser Leu Ala Asp Tyr Trp Lys	
290 295 300	
tgc act tca tgc aat gaa atg aat ccc ccc ctt cca tca cat tgc aac	960
Cys Thr Ser Cys Asn Glu Met Asn Pro Pro Leu Pro Ser His Cys Asn	
305 310 315 320	
aga tgt tgg gcc ctt cgt gag aat tgg ctt cct gaa gat aaa ggg aaa	1008
Arg Cys Trp Ala Leu Arg Glu Asn Trp Leu Pro Glu Asp Lys Gly Lys	
325 330 335	
gat aaa ggg gaa atc tct gag aaa gcc aaa ctg gaa aac tca aca caa	1056
Asp Lys Gly Glu Ile Ser Glu Lys Ala Lys Leu Glu Asn Ser Thr Gln	
340 345 350	
gct gaa gag ggc ttt gat gtt cct gat tgt aaa aaa act ata gtg aat	1104
Ala Glu Glu Gly Phe Asp Val Pro Asp Cys Lys Lys Thr Ile Val Asn	

355	360	365	
gat tcc aga gag tca tgt gtt gag gaa aat gat gat aaa att aca caa			1152
Asp Ser Arg Glu Ser Cys Val Glu Glu Asn Asp Asp Lys Ile Thr Gln			
370	375	380	
gct tca caa tca caa gaa agt gaa gac tat tct cag cca tca act tct			1200
Ala Ser Gln Ser Gln Glu Ser Glu Asp Tyr Ser Gln Pro Ser Thr Ser			
385	390	395	400
agt agc att att tat agc agc caa gaa gat gtg aaa gag ttt gaa agg			1248
Ser Ser Ile Ile Tyr Ser Ser Gln Glu Asp Val Lys Glu Phe Glu Arg			
405	410		415
gaa gaa acc caa gac aaa gaa gag agt gtg gaa tct agt ttg ccc ctt			1296
Glu Glu Thr Gln Asp Lys Glu Glu Ser Val Glu Ser Ser Leu Pro Leu			
420	425		430
aat gcc att gaa cct tgt gtg att tgt caa ggt cga cct aaa aat ggt			1344
Asn Ala Ile Glu Pro Cys Val Ile Cys Gln Gly Arg Pro Lys Asn Gly			
435	440		445
tgc att gtc cat ggc aaa aca gga cat ctt atg gcc tgc ttt aca tgt			1392
Cys Ile Val His Gly Lys Thr Gly His Leu Met Ala Cys Phe Thr Cys			
450	455		460
gca aag aag cta aag aaa agg aat aag ccc tgc cca gta tgt aga caa			1440
Ala Lys Lys Leu Lys Lys Arg Asn Lys Pro Cys Pro Val Cys Arg Gln			
465	470		475
cca att caa atg att gtg cta act tat ttc ccc tag			1476
Pro Ile Gln Met Ile Val Leu Thr Tyr Phe Pro			
485			490

&lt;210&gt; 15

&lt;211&gt; 491

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 15

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1	5	10	15

Ser Gln Ile Pro Ala Ser Glu Gln Glu Thr Leu Val Arg Pro Lys Pro			
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Leu Leu Leu Lys Leu Leu Lys Ser Val Gly Ala Gln Lys Asp Thr Tyr			
35	40		45

Thr Met Lys Glu Val Leu Phe Tyr Leu Gly Gln Tyr Ile Met Thr Lys			
50	55		60

Arg Leu Tyr Asp Glu Lys Gln Gln His Ile Val Tyr Cys Ser Asn Asp			
65	70	75	80

Leu Leu Gly Asp Leu Phe Gly Val Pro Ser Phe Ser Val Lys Glu His			
85	90		95



Arg Lys Ile Tyr Thr Met Ile Tyr Arg Asn Leu Val Val Val Asn Gln  
 100 105 110  
 Gln Glu Ser Ser Asp Ser Gly Thr Ser Val Ser Glu Asn Arg Cys His  
 115 120 125  
 Leu Glu Gly Gly Ser Asp Gln Lys Asp Leu Val Gln Glu Leu Gln Glu  
 130 135 140  
 Glu Lys Pro Ser Ser Ser His Leu Val Ser Arg Pro Ser Thr Ser Ser  
 145 150 155 160  
 Arg Arg Arg Ala Ile Ser Glu Thr Glu Glu Asn Ser Asp Glu Leu Ser  
 165 170 175  
 Gly Glu Arg Gln Arg Lys Arg His Lys Ser Asp Ser Ile Ser Leu Ser  
 180 185 190  
 Phe Asp Glu Ser Leu Ala Leu Cys Val Ile Arg Glu Ile Cys Cys Glu  
 195 200 205  
 Arg Ser Ser Ser Ser Glu Ser Thr Gly Thr Pro Ser Asn Pro Asp Leu  
 210 215 220  
 Asp Ala Gly Val Ser Glu His Ser Gly Asp Trp Leu Asp Gln Asp Ser  
 225 230 235 240  
 Val Ser Asp Gln Phe Ser Val Glu Phe Glu Val Glu Ser Leu Asp Ser  
 245 250 255  
 Glu Asp Tyr Ser Leu Ser Glu Glu Gly Gln Glu Leu Ser Asp Glu Asp  
 260 265 270  
 Asp Glu Val Tyr Gln Val Thr Val Tyr Gln Ala Gly Glu Ser Asp Thr  
 275 280 285  
 Asp Ser Phe Glu Glu Asp Pro Glu Ile Ser Leu Ala Asp Tyr Trp Lys  
 290 295 300  
 Cys Thr Ser Cys Asn Glu Met Asn Pro Pro Leu Pro Ser His Cys Asn  
 305 310 315 320  
 Arg Cys Trp Ala Leu Arg Glu Asn Trp Leu Pro Glu Asp Lys Gly Lys  
 325 330 335  
 Asp Lys Gly Glu Ile Ser Glu Lys Ala Lys Leu Glu Asn Ser Thr Gln  
 340 345 350  
 Ala Glu Glu Gly Phe Asp Val Pro Asp Cys Lys Lys Thr Ile Val Asn  
 355 360 365  
 Asp Ser Arg Glu Ser Cys Val Glu Glu Asn Asp Asp Lys Ile Thr Gln  
 370 375 380  
 Ala Ser Gln Ser Gln Glu Ser Glu Asp Tyr Ser Gln Pro Ser Thr Ser  
 385 390 395 400  
 Ser Ser Ile Ile Tyr Ser Ser Gln Glu Asp Val Lys Glu Phe Glu Arg

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ttccgggtca ctgcc atg gag gag ccg cag tca gat cct agc gtc gag ccc 171
      Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro
      1          5          10
cct ctg agt cag gaa aca ttt tca gac cta tgg aaa cta ctt cct gaa 219
Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Leu Leu Pro Glu
      15          20          25
aac aac gtt ctg tcc ccc ttg ccg tcc caa gca atg gat gat ttg atg 267
Asn Asn Val Leu Ser Pro Leu Pro Ser Gln Ala Met Asp Asp Leu Met
      30          35          40
ctg tcc ccg gac gat att gaa caa tgg ttc act gaa gac cca ggt cca 315
Leu Ser Pro Asp Asp Ile Glu Gln Trp Phe Thr Glu Asp Pro Gly Pro
      45          50          55          60
gat gaa gct ccc aga atg cca gag gct gct ccc ccc gtg gcc cct gca 363
Asp Glu Ala Pro Arg Met Pro Glu Ala Ala Pro Pro Val Ala Pro Ala
      65          70          75
cca gca gct cct aca ccg gcg gcc cct gca cca gcc ccc tcc tgg ccc 411
Pro Ala Ala Pro Thr Pro Ala Ala Pro Ala Pro Ala Pro Ser Trp Pro
      80          85          90

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ctg tca tct tct gtc cct tcc cag aaa acc tac cag ggc agc tac ggt	459
Leu Ser Ser Ser Val Pro Ser Gln Lys Thr Tyr Gln Gly Ser Tyr Gly	
95 100 105	
ttc cgt ctg ggc ttc ttg cat tct ggg aca gcc aag tct gtg act tgc	507
Phe Arg Leu Gly Phe Leu His Ser Gly Thr Ala Lys Ser Val Thr Cys	
110 115 120	
acg tac tcc cct gcc ctc aac aag atg ttt tgc caa ctg gcc aag acc	555
Thr Tyr Ser Pro Ala Leu Asn Lys Met Phe Cys Gln Leu Ala Lys Thr	
125 130 135 140	
tgc cct gtg cag ctg tgg gtt gat tcc aca ccc ccg ccc ggc acc cgc	603
Cys Pro Val Gln Leu Trp Val Asp Ser Thr Pro Pro Pro Gly Thr Arg	
145 150 155	
gtc cgc gcc atg gcc atc tac aag cag tca cag cac atg acg gag gtt	651
Val Arg Ala Met Ala Ile Tyr Lys Gln Ser Gln His Met Thr Glu Val	
160 165 170	
gtg agg cgc tgc ccc cac cat gag cgc tgc tca gat agc gat ggt ctg	699
Val Arg Arg Cys Pro His His Glu Arg Cys Ser Asp Ser Asp Gly Leu	
175 180 185	
gcc cct cct cag cat ctt atc cga gtg gaa gga aat ttg cgt gtg gag	747
Ala Pro Pro Gln His Leu Ile Arg Val Glu Gly Asn Leu Arg Val Glu	
190 195 200	
tat ttg gat gac aga aac act ttt cga cat agt gtg gtg gtg ccc tat	795
Tyr Leu Asp Asp Arg Asn Thr Phe Arg His Ser Val Val Val Pro Tyr	
205 210 215 220	
gag ccg cct gag gtt ggc tct gac tgt acc acc atc cac tac aac tac	843
Glu Pro Pro Glu Val Gly Ser Asp Cys Thr Thr Ile His Tyr Asn Tyr	
225 230 235	
atg tgt aac agt tcc tgc atg ggc ggc atg aac cgg agg ccc atc ctc	891
Met Cys Asn Ser Ser Cys Met Gly Gly Met Asn Arg Arg Pro Ile Leu	
240 245 250	
acc atc atc aca ctg gaa gac tcc agt ggt aat cta ctg gga cgg aac	939
Thr Ile Ile Thr Leu Glu Asp Ser Ser Gly Asn Leu Leu Gly Arg Asn	
255 260 265	
agc ttt gag gtg cgt gtt tgt gcc tgt cct ggg aga gac cgg cgc aca	987
Ser Phe Glu Val Arg Val Cys Ala Cys Pro Gly Arg Asp Arg Arg Thr	
270 275 280	
gag gaa gag aat ctc cgc aag aaa ggg gag cct cac cac gag ctg ccc	1035
Glu Glu Glu Asn Leu Arg Lys Lys Gly Glu Pro His His Glu Leu Pro	
285 290 295 300	
cca ggg agc act aag cga gca ctg ccc aac aac acc agc tcc tct ccc	1083
Pro Gly Ser Thr Lys Arg Ala Leu Pro Asn Asn Thr Ser Ser Ser Pro	
305 310 315	
cag cca aag aag aaa cca ctg gat gga gaa tat ttc acc ctt cag atc	1131
Gln Pro Lys Lys Lys Pro Leu Asp Gly Glu Tyr Phe Thr Leu Gln Ile	

320	325	330	
cgt ggg cgt gag cgc ttc gag atg ttc cga gag ctg aat gag gcc ttg			1179
Arg Gly Arg Glu Arg Phe Glu Met Phe Arg Glu Leu Asn Glu Ala Leu			
335	340	345	
gaa ctc aag gat gcc cag gct ggg aag gag cca ggg ggg agc agg gct			1227
Glu Leu Lys Asp Ala Gln Ala Gly Lys Glu Pro Gly Gly Ser Arg Ala			
350	355	360	
cac tcc agc cac ctg aag tcc aaa aag ggt cag tct acc tcc cgc cat			1275
His Ser Ser His Leu Lys Ser Lys Lys Gly Gln Ser Thr Ser Arg His			
365	370	375	380
aaa aaa ctc atg ttc aag aca gaa ggg cct gac tca gac tga			1317
Lys Lys Leu Met Phe Lys Thr Glu Gly Pro Asp Ser Asp			
385	390		

<210> 17  
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 <212> PRT  
 <213> Homo sapiens

<400> 17  
 Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln  
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 Glu Thr Phe Ser Asp Leu Trp Lys Leu Leu Pro Glu Asn Asn Val Leu  
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 Ser Pro Leu Pro Ser Gln Ala Met Asp Asp Leu Met Leu Ser Pro Asp  
 35 40 45  
 Asp Ile Glu Gln Trp Phe Thr Glu Asp Pro Gly Pro Asp Glu Ala Pro  
 50 55 60  
 Arg Met Pro Glu Ala Ala Pro Pro Val Ala Pro Ala Pro Ala Ala Pro  
 65 70 75 80  
 Thr Pro Ala Ala Pro Ala Pro Ala Pro Ser Trp Pro Leu Ser Ser Ser  
 85 90 95  
 Val Pro Ser Gln Lys Thr Tyr Gln Gly Ser Tyr Gly Phe Arg Leu Gly  
 100 105 110  
 Phe Leu His Ser Gly Thr Ala Lys Ser Val Thr Cys Thr Tyr Ser Pro  
 115 120 125  
 Ala Leu Asn Lys Met Phe Cys Gln Leu Ala Lys Thr Cys Pro Val Gln  
 130 135 140  
 Leu Trp Val Asp Ser Thr Pro Pro Pro Gly Thr Arg Val Arg Ala Met  
 145 150 155 160  
 Ala Ile Tyr Lys Gln Ser Gln His Met Thr Glu Val Val Arg Arg Cys  
 165 170 175

Pro His His Glu Arg Cys Ser Asp Ser Asp Gly Leu Ala Pro Pro Gln  
 180 185 190  
 His Leu Ile Arg Val Glu Gly Asn Leu Arg Val Glu Tyr Leu Asp Asp  
 195 200 205  
 Arg Asn Thr Phe Arg His Ser Val Val Val Pro Tyr Glu Pro Pro Glu  
 210 215 220  
 Val Gly Ser Asp Cys Thr Thr Ile His Tyr Asn Tyr Met Cys Asn Ser  
 225 230 235 240  
 Ser Cys Met Gly Gly Met Asn Arg Arg Pro Ile Leu Thr Ile Ile Thr  
 245 250 255  
 Leu Glu Asp Ser Ser Gly Asn Leu Leu Gly Arg Asn Ser Phe Glu Val  
 260 265 270  
 Arg Val Cys Ala Cys Pro Gly Arg Asp Arg Arg Thr Glu Glu Glu Asn  
 275 280 285  
 Leu Arg Lys Lys Gly Glu Pro His His Glu Leu Pro Pro Gly Ser Thr  
 290 295 300  
 Lys Arg Ala Leu Pro Asn Asn Thr Ser Ser Ser Pro Gln Pro Lys Lys  
 305 310 315 320  
 Lys Pro Leu Asp Gly Glu Tyr Phe Thr Leu Gln Ile Arg Gly Arg Glu  
 325 330 335  
 Arg Phe Glu Met Phe Arg Glu Leu Asn Glu Ala Leu Glu Leu Lys Asp  
 340 345 350  
 Ala Gln Ala Gly Lys Glu Pro Gly Gly Ser Arg Ala His Ser Ser His  
 355 360 365  
 Leu Lys Ser Lys Lys Gly Gln Ser Thr Ser Arg His Lys Lys Leu Met  
 370 375 380  
 Phe Lys Thr Glu Gly Pro Asp Ser Asp  
 385 390

&lt;210&gt; 18

&lt;211&gt; 579

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1) .. (576)

&lt;223&gt; Human Bax

&lt;400&gt; 18

atg gac ggg tcc ggg gag cag ccc aga ggc ggg ggg ccc acc agc tct 48  
 Met Asp Gly Ser Gly Glu Gln Pro Arg Gly Gly Gly Pro Thr Ser Ser  
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gag cag atc atg aag aca ggg gcc ctt ttg ctt cag ggt ttc atc cag 96  
 Glu Gln Ile Met Lys Thr Gly Ala Leu Leu Leu Gln Gly Phe Ile Gln  
 20 25 30

gat cga gca ggg cga atg ggg ggg gag gca ccc gag ctg gcc ctg gac 144  
 Asp Arg Ala Gly Arg Met Gly Gly Glu Ala Pro Glu Leu Ala Leu Asp  
 35 40 45

ccg gtg cct cag gat gcg tcc acc aag aag ctg agc gag tgt ctc aag 192  
 Pro Val Pro Gln Asp Ala Ser Thr Lys Lys Leu Ser Glu Cys Leu Lys  
 50 55 60

cgc atc ggg gac gaa ctg gac agt aac atg gag ctg cag agg atg att 240  
 Arg Ile Gly Asp Glu Leu Asp Ser Asn Met Glu Leu Gln Arg Met Ile  
 65 70 75 80

gcc gcc gtg gac aca gac tcc ccc cga gag gtc ttt ttc cga gtg gca 288  
 Ala Ala Val Asp Thr Asp Ser Pro Arg Glu Val Phe Phe Arg Val Ala  
 85 90 95

gct gac atg ttt tct gac ggc aac ttc aac tgg ggc cgg gtt gtc gcc 336  
 Ala Asp Met Phe Ser Asp Gly Asn Phe Asn Trp Gly Arg Val Val Ala  
 100 105 110

ctt ttc tac ttt gcc agc aaa ctg gtg ctc aag gcc ctg tgc acc aag 384  
 Leu Phe Tyr Phe Ala Ser Lys Leu Val Leu Lys Ala Leu Cys Thr Lys  
 115 120 125

gtg ccg gaa ctg atc aga acc atc atg ggc tgg aca ttg gac ttc ctc 432  
 Val Pro Glu Leu Ile Arg Thr Ile Met Gly Trp Thr Leu Asp Phe Leu  
 130 135 140

cgg gag cgg ctg ttg ggc tgg atc caa gac cag ggt ggt tgg gac ggc 480  
 Arg Glu Arg Leu Leu Gly Trp Ile Gln Asp Gln Gly Gly Trp Asp Gly  
 145 150 155 160

ctc ctc tcc tac ttt ggg acg ccc acg tgg cag acc gtg acc atc ttt 528  
 Leu Leu Ser Tyr Phe Gly Thr Pro Thr Trp Gln Thr Val Thr Ile Phe  
 165 170 175

gtg gcg gga gtg ctc acc gcc tcg ctc acc atc tgg aag aag atg ggc 576  
 Val Ala Gly Val Leu Thr Ala Ser Leu Thr Ile Trp Lys Lys Met Gly  
 180 185 190

tga 579

&lt;210&gt; 19

&lt;211&gt; 192

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 19

Met Asp Gly Ser Gly Glu Gln Pro Arg Gly Gly Gly Pro Thr Ser Ser  
 1 5 10 15

Glu Gln Ile Met Lys Thr Gly Ala Leu Leu Leu Gln Gly Phe Ile Gln  
 20 25 30

Asp Arg Ala Gly Arg Met Gly Gly Glu Ala Pro Glu Leu Ala Leu Asp  
 35 40 45

Pro Val Pro Gln Asp Ala Ser Thr Lys Lys Leu Ser Glu Cys Leu Lys  
 50 55 60

Arg Ile Gly Asp Glu Leu Asp Ser Asn Met Glu Leu Gln Arg Met Ile  
 65 70 75 80

Ala Ala Val Asp Thr Asp Ser Pro Arg Glu Val Phe Phe Arg Val Ala  
 85 90 95

Ala Asp Met Phe Ser Asp Gly Asn Phe Asn Trp Gly Arg Val Val Ala  
 100 105 110

Leu Phe Tyr Phe Ala Ser Lys Leu Val Leu Lys Ala Leu Cys Thr Lys  
 115 120 125

Val Pro Glu Leu Ile Arg Thr Ile Met Gly Trp Thr Leu Asp Phe Leu  
 130 135 140

Arg Glu Arg Leu Leu Gly Trp Ile Gln Asp Gln Gly Gly Trp Asp Gly  
 145 150 155 160

Leu Leu Ser Tyr Phe Gly Thr Pro Thr Trp Gln Thr Val Thr Ile Phe  
 165 170 175

Val Ala Gly Val Leu Thr Ala Ser Leu Thr Ile Trp Lys Lys Met Gly  
 180 185 190

<210> 20

<211> 507

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1) .. (504)

<223> Human Bad

<400> 20

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 Met Phe Gln Ile Pro Glu Phe Glu Pro Ser Glu Gln Glu Asp Ser Ser  
 1 5 10 15

tct gca gag agg ggc ctg ggc ccc agc ccc gca ggg gac ggg ccc tca 96  
 Ser Ala Glu Arg Gly Leu Gly Pro Ser Pro Ala Gly Asp Gly Pro Ser  
 20 25 30

ggc tcc ggc aag cat cat cgc cag gcc cca ggc ctc ctg tgg gac gcc 144  
 Gly Ser Gly Lys His His Arg Gln Ala Pro Gly Leu Leu Trp Asp Ala  
 35 40 45

agt cac cag cag gag cag cca acc agc agc agc cat cat gga ggc gct 192  
 Ser His Gln Gln Glu Gln Pro Thr Ser Ser Ser His His Gly Gly Ala  
 50 55 60

20

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ggg gct gtg gag atc cgg agt cgc cac agc tcc tac ccc gcg ggg acg      240
Gly Ala Val Glu Ile Arg Ser Arg His Ser Ser Tyr Pro Ala Gly Thr
 65                      70                      75                      80

gag gac gac gaa ggg atg ggg gag gag ccc agc ccc ttt cgg ggc cgc      288
Glu Asp Asp Glu Gly Met Gly Glu Glu Pro Ser Pro Phe Arg Gly Arg
                      85                      90                      95

tcg cgc tcg gcg ccc ccc aac ctc tgg gca gca cag cgc tat ggc cgc      336
Ser Arg Ser Ala Pro Pro Asn Leu Trp Ala Ala Gln Arg Tyr Gly Arg
                100                      105                      110

gag ctc cgg agg atg agt gac gag ttt gtg gac tcc ttt aag aag gga      384
Glu Leu Arg Arg Met Ser Asp Glu Phe Val Asp Ser Phe Lys Lys Gly
                115                      120                      125

ctt cct cgc ccg aag agc gcg ggc aca gca acg cag atg cgg caa agc      432
Leu Pro Arg Pro Lys Ser Ala Gly Thr Ala Thr Gln Met Arg Gln Ser
                130                      135                      140

tcc agc tgg acg cga gtc ttc cag tcc tgg tgg gat cgg aac ttg ggc      480
Ser Ser Trp Thr Arg Val Phe Gln Ser Trp Trp Asp Arg Asn Leu Gly
145                      150                      155                      160

agg gga agc tcc gcc ccc tcc cag tga                                  507
Arg Gly Ser Ser Ala Pro Ser Gln
                165

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<210> 21  
 <211> 168  
 <212> PRT  
 <213> Homo sapiens

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<400> 21
Met Phe Gln Ile Pro Glu Phe Glu Pro Ser Glu Gln Glu Asp Ser Ser
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Ser Ala Glu Arg Gly Leu Gly Pro Ser Pro Ala Gly Asp Gly Pro Ser
                20                      25                      30

Gly Ser Gly Lys His His Arg Gln Ala Pro Gly Leu Leu Trp Asp Ala
                35                      40                      45

Ser His Gln Gln Glu Gln Pro Thr Ser Ser Ser His His Gly Gly Ala
                50                      55                      60

Gly Ala Val Glu Ile Arg Ser Arg His Ser Ser Tyr Pro Ala Gly Thr
 65                      70                      75                      80

Glu Asp Asp Glu Gly Met Gly Glu Glu Pro Ser Pro Phe Arg Gly Arg
                      85                      90                      95

Ser Arg Ser Ala Pro Pro Asn Leu Trp Ala Ala Gln Arg Tyr Gly Arg
                100                      105                      110

Glu Leu Arg Arg Met Ser Asp Glu Phe Val Asp Ser Phe Lys Lys Gly
                115                      120                      125

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Arg Gly Ser Ser Ala Pro Ser Gln  
165

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<220>
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<222> (34) .. (750)
<223> Human BCL-2
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g	g	t	a	c	g	a	t	a	g	a	a	a	a	a	a							
Gly	Tyr	Asp	Asn	Arg	Glu	Ile	Val	Met	Lys	Tyr	Ile	His	Tyr	Lys	Leu							
				10					15					20								
t	c	g	a	g	g	a	t	a	a	a	a	a	a	a	a							
Ser	Gln	Arg	Gly	Tyr	Glu	Trp	Asp	Ala	Gly	Asp	Val	Gly	Ala	Ala	Pro							
				25					30					35								
c	c	g	g	g	c	c	c	c	c	c	c	c	c	c	c							
Pro	Gly	Ala	Ala	Pro	Ala	Pro	Gly	Ile	Phe	Ser	Ser	Gln	Pro	Gly	His							
				40					45					50								
a	c	c	c	a	g	c	a	t	c	c	g	a	c	c	c							
Thr	Pro	His	Thr	Ala	Ala	Ser	Arg	Asp	Pro	Val	Ala	Arg	Thr	Ser	Pro							
				60					65					70								
c	t	g	a	a	c	c	c	g	c	c	c	c	c	c	c							
Leu	Gln	Thr	Pro	Ala	Ala	Pro	Gly	Ala	Ala	Ala	Gly	Pro	Ala	Leu	Ser							
				75					80					85								
c	c	g	g	c	c	t	g	t	c	c	c	c	c	c	c							
Pro	Val	Pro	Pro	Val	Val	His	Leu	Thr	Leu	Arg	Gln	Ala	Gly	Asp	Asp							
				90					95					100								
t	t	c	c	g	c	a	c	c	c	c	c	c	c	c	c							
Phe	Ser	Arg	Arg	Tyr	Arg	Arg	Asp	Phe	Ala	Glu	Met	Ser	Arg	Gln	Leu							
				105					110					115								
c	a	c	c	t	a	c	c	c	c	c	c	c	c	c	c							
His	Leu	Thr	Pro	Phe	Thr	Ala	Arg	Gly	Arg	Phe	Ala	Thr	Val	Val	Glu							
				120					125					130								
																135						

gag ctc ttc agg gac ggg gtg aac tgg ggg agg att gtg gcc ttc ttt 486  
 Glu Leu Phe Arg Asp Gly Val Asn Trp Gly Arg Ile Val Ala Phe Phe  
 140 145 150

gag ttc ggt ggg gtc atg tgt gtg gag agc gtc aac cgg gag atg tcg 534  
 Glu Phe Gly Gly Val Met Cys Val Glu Ser Val Asn Arg Glu Met Ser  
 155 160 165

ccc ctg gtg gac aac atc gcc ctg tgg atg act gag tac ctg aac cgg 582  
 Pro Leu Val Asp Asn Ile Ala Leu Trp Met Thr Glu Tyr Leu Asn Arg  
 170 175 180

cac ctg cac acc tgg atc cag gat aac gga ggc tgg gat gcc ttt gtg 630  
 His Leu His Thr Trp Ile Gln Asp Asn Gly Gly Trp Asp Ala Phe Val  
 185 190 195

gaa ctg tac ggc ccc agc atg cgg cct ctg ttt gat ttc tcc tgg ctg 678  
 Glu Leu Tyr Gly Pro Ser Met Arg Pro Leu Phe Asp Phe Ser Trp Leu  
 200 205 210 215

tct ctg aag act ctg ctc agt ttg gcc ctg gtg gga gct tgc atc acc 726  
 Ser Leu Lys Thr Leu Leu Ser Leu Ala Leu Val Gly Ala Cys Ile Thr  
 220 225 230

ctg ggt gcc tat ctg ggc cac aag tgaagtcaac atgcctgccc caaacaata 780  
 Leu Gly Ala Tyr Leu Gly His Lys  
 235

tgcaaaagggt tcactaaagc agtagaaata atatgcattg tcagtgatgt tccatgaaac 840  
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ttaaacaata aatgtgcagt tttaactaac aggatattta atgacaacct tctggttgg 5100  
agggacatct gtttctaaat gtttattatg tacaatacag aaaaaattt tataaaatta 5160

agcaatgtga aactgaattg gagagtgata atacaagtcc tttagtctta cccagtgaat 5220  
 cattctgttc catgtctttg gacaaccatg accttggaca atcatgaaat atgcatctca 5280  
 ctggatgcaa agaaaatcag atggagcatg aatgggtactg taccgggttca tctggactgc 5340  
 cccagaaaaa taacttcaag caaacatcct atcaacaaca aggttggttct gcataccaag 5400  
 ctgagcacag aagatgggaa cactgggtgga ggatgggaaag gctcgctcaa tcaagaaaat 5460  
 tctgagacta ttaataaata agactgtagt gtagatactg agtaaatacca tgcacctaaa 5520  
 ccttttggaa aatctgccgt gggccctcca gatagctcat ttcattaagt tttccctcc 5580  
 aaggtagaat ttgcaagagt gacagtggat tgcatttctt ttggggaagc tttcttttgg 5640  
 tggttttgtt tattatacct tcttaagttt tcaaccaagg tttgcttttg ttttgagtta 5700  
 ctgggggttat ttttgtttta aataaaaata agtgtacaat aagtgttttt gtattgaaag 5760  
 cttttgttat caagattttc atacttttac cttccatggc tctttttaag attgatactt 5820  
 ttaagaggtg gctgatattc tgcaacactg tacacataaa aaatacggta aggatacttt 5880  
 acatggttaa ggtaaagtaa gtctccagtt ggccaccatt agctataatg gcactttgtt 5940  
 tgtgttgttg gaaaaagtca cattgccatt aaactttcct tgtctgtcta gttaatatgt 6000  
 tgaagaaaaa taaagtacag tgtgagatac tg 6032

&lt;210&gt; 23

&lt;211&gt; 239

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 23

Met Ala His Ala Gly Arg Thr Gly Tyr Asp Asn Arg Glu Ile Val Met  
 1 5 10 15

Lys Tyr Ile His Tyr Lys Leu Ser Gln Arg Gly Tyr Glu Trp Asp Ala  
 20 25 30

Gly Asp Val Gly Ala Ala Pro Pro Gly Ala Ala Pro Ala Pro Gly Ile  
 35 40 45

Phe Ser Ser Gln Pro Gly His Thr Pro His Thr Ala Ala Ser Arg Asp  
 50 55 60

Pro Val Ala Arg Thr Ser Pro Leu Gln Thr Pro Ala Ala Pro Gly Ala  
 65 70 75 80

Ala Ala Gly Pro Ala Leu Ser Pro Val Pro Pro Val Val His Leu Thr  
 85 90 95

Leu Arg Gln Ala Gly Asp Asp Phe Ser Arg Arg Tyr Arg Arg Asp Phe  
 100 105 110

26

Ala Glu Met Ser Arg Gln Leu His Leu Thr Pro Phe Thr Ala Arg Gly  
 115 120 125

Arg Phe Ala Thr Val Val Glu Glu Leu Phe Arg Asp Gly Val Asn Trp  
 130 135 140

Gly Arg Ile Val Ala Phe Phe Glu Phe Gly Gly Val Met Cys Val Glu  
 145 150 155 160

Ser Val Asn Arg Glu Met Ser Pro Leu Val Asp Asn Ile Ala Leu Trp  
 165 170 175

Met Thr Glu Tyr Leu Asn Arg His Leu His Thr Trp Ile Gln Asp Asn  
 180 185 190

Gly Gly Trp Asp Ala Phe Val Glu Leu Tyr Gly Pro Ser Met Arg Pro  
 195 200 205

Leu Phe Asp Phe Ser Trp Leu Ser Leu Lys Thr Leu Leu Ser Leu Ala  
 210 215 220

Leu Val Gly Ala Cys Ile Thr Leu Gly Ala Tyr Leu Gly His Lys  
 225 230 235

&lt;210&gt; 24

&lt;211&gt; 1561

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (70)..(1413)

&lt;223&gt; Description of Artificial Sequence:

Beta-TRCP.N/SKP2.C hybrid cDNA and protein

&lt;400&gt; 24

tgcgttggct gcggcctggc accaaagggg cggccccggc ggagagcgga cccagtggcc 60

tggcgatt atg gac ccg gcc gag gcg gtg ctg caa gag aag gca ctc aag 111  
 Met Asp Pro Ala Glu Ala Val Leu Gln Glu Lys Ala Leu Lys  
 1 5 10

ttt atg aca tac aac agc tgt gcc aga ctc tgc tta aac caa gaa aca 159  
 Phe Met Thr Tyr Asn Ser Cys Ala Arg Leu Cys Leu Asn Gln Glu Thr  
 15 20 25 30

gta tgt tta gca agc act gct atg aag act gag aat tgt gtg gcc aaa 207  
 Val Cys Leu Ala Ser Thr Ala Met Lys Thr Glu Asn Cys Val Ala Lys  
 35 40 45

aca aaa ctt gcc aat ggc act tcc agt atg att gtg ccc aag caa cgg 255  
 Thr Lys Leu Ala Asn Gly Thr Ser Ser Met Ile Val Pro Lys Gln Arg  
 50 55 60

aaa ctc tca gca agc tat gaa aag gaa aag gaa ctg tgt gtc aaa tac 303  
 Lys Leu Ser Ala Ser Tyr Glu Lys Glu Lys Glu Leu Cys Val Lys Tyr

27

65	70	75	
ttt gag cag tgg tca gag tca gat caa gtg gaa ttt gtg gaa cat ctt			351
Phe Glu Gln Trp Ser Glu Ser Asp Gln Val Glu Phe Val Glu His Leu			
80	85	90	
ata tcc caa atg tgt cat tac caa cat ggg cac ata aac tcg tat ctt			399
Ile Ser Gln Met Cys His Tyr Gln His Gly His Ile Asn Ser Tyr Leu			
95	100	105	110
aaa cct atg ttg cag aga gat ttc ata act gct ctg cca gct cgg gga			447
Lys Pro Met Leu Gln Arg Asp Phe Ile Thr Ala Leu Pro Ala Arg Gly			
115	120	125	
ttg gat cat atc gct gag aac att ctg tca tac ctg gat gcc aaa tca			495
Leu Asp His Ile Ala Glu Asn Ile Leu Ser Tyr Leu Asp Ala Lys Ser			
130	135	140	
cta tgt gct gct gaa ctt gtg tgc aag gaa tgg tac cga gtg acc tct			543
Leu Cys Ala Ala Glu Leu Val Cys Lys Glu Trp Tyr Arg Val Thr Ser			
145	150	155	
gat ggc atg ctg tgg aag aag ctt atc gag aga atg gtc agg aca gat			591
Asp Gly Met Leu Trp Lys Lys Leu Ile Glu Arg Met Val Arg Thr Asp			
160	165	170	
tct ctg tgg aga gcc atg gtg tct caa ggg gtg att gcc ttc cgc tgc			639
Ser Leu Trp Arg Ala Met Val Ser Gln Gly Val Ile Ala Phe Arg Cys			
175	180	185	190
cca cga tca ttt atg gac caa cca ttg gct gaa cat ttc agc cct ttt			687
Pro Arg Ser Phe Met Asp Gln Pro Leu Ala Glu His Phe Ser Pro Phe			
195	200	205	
cgt gta cag gac atg gac cta tgc aac tca gtt ata gaa gtg tcc acc			735
Arg Val Gln Asp Met Asp Leu Ser Asn Ser Val Ile Glu Val Ser Thr			
210	215	220	
ctc cac ggc ata ctg tct cag tgt tcc aag ttg cag aat cta agc ctg			783
Leu His Gly Ile Leu Ser Gln Cys Ser Lys Leu Gln Asn Leu Ser Leu			
225	230	235	
gaa ctg cgg ctt tcg gat ccc att gtc aat act ctc gca aaa aac tca			831
Glu Leu Arg Leu Ser Asp Pro Ile Val Asn Thr Leu Ala Lys Asn Ser			
240	245	250	
aat tta gtg cga ctt aac ctt cct ggg tgt cct gga ttc cct aaa ttt			879
Asn Leu Val Arg Leu Asn Leu Pro Gly Cys Pro Gly Phe Pro Lys Phe			
255	260	265	270
ccc ctg cag act ttc cta agc agc tgt ccc aga ctg gat gag ctg aac			927
Pro Leu Gln Thr Phe Leu Ser Ser Cys Pro Arg Leu Asp Glu Leu Asn			
275	280	285	
ctc tcc tgg tgt ttt aat ttc act gaa aag cat gta cag gtg gct gtt			975
Leu Ser Trp Cys Phe Asn Phe Thr Glu Lys His Val Gln Val Ala Val			
290	295	300	

28

gcg cat gtc tca gag acc atg acc cag ctg aat cta agc ggc tac aga 1023  
 Ala His Val Ser Glu Thr Met Thr Gln Leu Asn Leu Ser Gly Tyr Arg  
 305 310 315

aag aat ctc cag aaa tca gat ctc tct act tta gtt aga aga tgc ccc 1071  
 Lys Asn Leu Gln Lys Ser Asp Leu Ser Thr Leu Val Arg Arg Cys Pro  
 320 325 330

aat ctt gtc cat cta gac tta agt aat agt gtc atg cta aag aat gac 1119  
 Asn Leu Val His Leu Asp Leu Ser Asn Ser Val Met Leu Lys Asn Asp  
 335 340 345 350

tgc ttt cag gaa ttt tcc cag ctc aac tac ctc caa cac cta tca ctc 1167  
 Cys Phe Gln Glu Phe Ser Gln Leu Asn Tyr Leu Gln His Leu Ser Leu  
 355 360 365

agt cgg tgc tat gat ata ata cct gaa act tta ctt gaa ctt gga gaa 1215  
 Ser Arg Cys Tyr Asp Ile Ile Pro Glu Thr Leu Leu Glu Leu Gly Glu  
 370 375 380

att ccc aca cta aaa aca cta caa gtt ttt gga atc gtg cca gat ggt 1263  
 Ile Pro Thr Leu Lys Thr Leu Gln Val Phe Gly Ile Val Pro Asp Gly  
 385 390 395

acc ctt caa ctg tta aag gaa gcc ctt cct cat cta cag att aat tgc 1311  
 Thr Leu Gln Leu Leu Lys Glu Ala Leu Pro His Leu Gln Ile Asn Cys  
 400 405 410

tcc cat ttc acc acc att gcc agg cca act att ggc aac aaa aag aac 1359  
 Ser His Phe Thr Thr Ile Ala Arg Pro Thr Ile Gly Asn Lys Lys Asn  
 415 420 425 430

cag gag ata tgg ggc atc aaa tgc cga ctg aca ctg caa aag ccc agt 1407  
 Gln Glu Ile Trp Gly Ile Lys Cys Arg Leu Thr Leu Gln Lys Pro Ser  
 435 440 445

tgt cta tgaagtattt attgcaggat ggtgtctctt ctttagaaca gggaaaatag 1463  
 Cys Leu

gcaggaagcc caattgctgg agtacttagc tagttttatt cttgggttttc ccttttgcct 1523

gtcattctgc aagtatacta gggagcccat tttgagag 1561

&lt;210&gt; 25

&lt;211&gt; 448

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;400&gt; 25

Met Asp Pro Ala Glu Ala Val Leu Gln Glu Lys Ala Leu Lys Phe Met  
 1 5 10 15

Thr Tyr Asn Ser Cys Ala Arg Leu Cys Leu Asn Gln Glu Thr Val Cys  
 20 25 30

Leu Ala Ser Thr Ala Met Lys Thr Glu Asn Cys Val Ala Lys Thr Lys  
 35 40 45



Leu Ala Asn Gly Thr Ser Ser Met Ile Val Pro Lys Gln Arg Lys Leu  
 50 55 60  
 Ser Ala Ser Tyr Glu Lys Glu Lys Glu Leu Cys Val Lys Tyr Phe Glu  
 65 70 75 80  
 Gln Trp Ser Glu Ser Asp Gln Val Glu Phe Val Glu His Leu Ile Ser  
 85 90 95  
 Gln Met Cys His Tyr Gln His Gly His Ile Asn Ser Tyr Leu Lys Pro  
 100 105 110  
 Met Leu Gln Arg Asp Phe Ile Thr Ala Leu Pro Ala Arg Gly Leu Asp  
 115 120 125  
 His Ile Ala Glu Asn Ile Leu Ser Tyr Leu Asp Ala Lys Ser Leu Cys  
 130 135 140  
 Ala Ala Glu Leu Val Cys Lys Glu Trp Tyr Arg Val Thr Ser Asp Gly  
 145 150 155 160  
 Met Leu Trp Lys Lys Leu Ile Glu Arg Met Val Arg Thr Asp Ser Leu  
 165 170 175  
 Trp Arg Ala Met Val Ser Gln Gly Val Ile Ala Phe Arg Cys Pro Arg  
 180 185 190  
 Ser Phe Met Asp Gln Pro Leu Ala Glu His Phe Ser Pro Phe Arg Val  
 195 200 205  
 Gln Asp Met Asp Leu Ser Asn Ser Val Ile Glu Val Ser Thr Leu His  
 210 215 220  
 Gly Ile Leu Ser Gln Cys Ser Lys Leu Gln Asn Leu Ser Leu Glu Leu  
 225 230 235 240  
 Arg Leu Ser Asp Pro Ile Val Asn Thr Leu Ala Lys Asn Ser Asn Leu  
 245 250 255  
 Val Arg Leu Asn Leu Pro Gly Cys Pro Gly Phe Pro Lys Phe Pro Leu  
 260 265 270  
 Gln Thr Phe Leu Ser Ser Cys Pro Arg Leu Asp Glu Leu Asn Leu Ser  
 275 280 285  
 Trp Cys Phe Asn Phe Thr Glu Lys His Val Gln Val Ala Val Ala His  
 290 295 300  
 Val Ser Glu Thr Met Thr Gln Leu Asn Leu Ser Gly Tyr Arg Lys Asn  
 305 310 315 320  
 Leu Gln Lys Ser Asp Leu Ser Thr Leu Val Arg Arg Cys Pro Asn Leu  
 325 330 335  
 Val His Leu Asp Leu Ser Asn Ser Val Met Leu Lys Asn Asp Cys Phe  
 340 345 350  
 Gln Glu Phe Ser Gln Leu Asn Tyr Leu Gln His Leu Ser Leu Ser Arg

355                                      360                                      365  
 Cys Tyr Asp Ile Ile Pro Glu Thr Leu Leu Glu Leu Gly Glu Ile Pro  
 370                                      375                                      380  
 Thr Leu Lys Thr Leu Gln Val Phe Gly Ile Val Pro Asp Gly Thr Leu  
 385                                      390                                      395                                      400  
 Gln Leu Leu Lys Glu Ala Leu Pro His Leu Gln Ile Asn Cys Ser His  
 405                                      410                                      415  
 Phe Thr Thr Ile Ala Arg Pro Thr Ile Gly Asn Lys Lys Asn Gln Glu  
 420                                      425                                      430  
 Ile Trp Gly Ile Lys Cys Arg Leu Thr Leu Gln Lys Pro Ser Cys Leu  
 435                                      440                                      445

<210> 26  
 <211> 1400  
 <212> DNA  
 <213> Homo sapiens

<400> 26  
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 ttaagaatat tttccttctt ggatatagta actttgtgcc gatgtgcaca gatttccaag 120  
 gcttggaaca tcttagccct ggatggaagc aactggcaaa gaatagatct ttttaacttt 180  
 caaatagatg tagagggtcg agtgggtgaa aatatctcga agcgatgcgg tggattcctg 240  
 aggaagctca gcttgcgagg ctgcattggt gttggggatt cctccttgaa gacctttgca 300  
 cagaactgcc gaaacattga acatttgaac ctcaatggat gcacaaaaat cactgacagc 360  
 acgtgttata gccttagcag attctgttcc aagctgaaac atctggatct gacctcctgt 420  
 gtgtctatta caaacagctc cttgaagggg atcagtgaag gctgccgaaa cctggagtag 480  
 ctgaacctct cttggtgtga tcagatcacg aaggatggca tcgaggcact ggtgcgaggt 540  
 tgtcgaggcc tgaaagccct gctcctgagg ggctgcacac agttagaaga tgaagctctg 600  
 aaacacattc agaattactg ccatgagctt gtgagcctca acttgcaagc ctgctcacgt 660  
 atcacggatg aagggtgtgg gcatgatgac aggggctgtc accggctaca ggctctctgc 720  
 ctttcggggt gcagcaacct cacagatgcc tctcttacag ccctggggtt gaactgtccg 780  
 cgactgcaaa ttttgagggc tgcccgatgc tccatttga ctgacgcagg ttttacactt 840  
 ttagctcgga attgccacga attggagaag atggatcttg aagaatgcat cctgataacc 900  
 gacagcacac tcatccagct ctccattcac tgcctaaac tgcaagccct gagcctgtcc 960  
 cactgtgaac tcatcacaga tgatgggata ctgcacctga gcaacagtag ctgtggccat 1020  
 gagaggctgc gggtagctgga gttggacaac tgctcctca tcaactgatg ggccctggaa 1080  
 cacctagaga actgccgagg cctggagcgc ctcgagctgt acgactgcca gcaggttacc 1140  
 cgtgcaggca tcaagcggat gcgggctcag ctccctcatg tcaaagtcca cgcctacttt 1200  
 gctcccgtca cccaccgac agcagtggca ggaagtggac agcgactgtg cagggtgctgt 1260  
 gtcattctct gacagcagct gcctgggccc aaggggtgat gaggcatacct ttcctctaga 1320  
 agacctgagt cttcctgacc gactccacca tcaccaatc tgttgattct ccattgggaa 1380  
 aggcatttac aggtaaaaga 1400

<210> 27  
 <211> 466  
 <212> PRT  
 <213> Homo sapiens

<400> 27  
 Met Val Phe Ser Asn Asn Asp Glu Gly Leu Ile Asn Lys Lys Leu Pro

1	5	10	15
Lys Glu Leu Leu Leu Arg Ile Phe Ser Phe Leu Asp Ile Val Thr Leu	20	25	30
Cys Arg Cys Ala Gln Ile Ser Lys Ala Trp Asn Ile Leu Ala Leu Asp	35	40	45
Gly Ser Asn Trp Gln Arg Ile Asp Leu Phe Asn Phe Gln Ile Asp Val	50	55	60
Glu Gly Arg Val Val Glu Asn Ile Ser Lys Arg Cys Gly Gly Phe Leu	65	70	75
Arg Lys Leu Ser Leu Arg Gly Cys Ile Gly Val Gly Asp Ser Ser Leu	85	90	95
Lys Thr Phe Ala Gln Asn Cys Arg Asn Ile Glu His Leu Asn Leu Asn	100	105	110
Gly Cys Thr Lys Ile Thr Asp Ser Thr Cys Tyr Ser Leu Ser Arg Phe	115	120	125
Cys Ser Lys Leu Lys His Leu Asp Leu Thr Ser Cys Val Ser Ile Thr	130	135	140
Asn Ser Ser Leu Lys Gly Ile Ser Glu Gly Cys Arg Asn Leu Glu Tyr	145	150	155
Leu Asn Leu Ser Trp Cys Asp Gln Ile Thr Lys Asp Gly Ile Glu Ala	165	170	175
Leu Val Arg Gly Cys Arg Gly Leu Lys Ala Leu Leu Leu Arg Gly Cys	180	185	190
Thr Gln Leu Glu Asp Glu Ala Leu Lys His Ile Gln Asn Tyr Cys His	195	200	205
Glu Leu Val Ser Leu Asn Leu Gln Ser Cys Ser Arg Ile Thr Asp Glu	210	215	220
Gly Val Val Gln Ile Cys Arg Gly Cys His Arg Leu Gln Ala Leu Cys	225	230	235
Leu Ser Gly Cys Ser Asn Leu Thr Asp Ala Ser Leu Thr Ala Leu Gly	245	250	255
Leu Asn Cys Pro Arg Leu Gln Ile Leu Glu Ala Ala Arg Cys Ser His	260	265	270
Leu Thr Asp Ala Gly Phe Thr Leu Leu Ala Arg Asn Cys His Glu Leu	275	280	285
Glu Lys Met Asp Leu Glu Glu Cys Ile Leu Ile Thr Asp Ser Thr Leu	290	295	300
Ile Gln Leu Ser Ile His Cys Pro Lys Leu Gln Ala Leu Ser Leu Ser	305	310	315
			320

His Cys Glu Leu Ile Thr Asp Asp Gly Ile Leu His Leu Ser Asn Ser  
 325 330 335  
 Thr Cys Gly His Glu Arg Leu Arg Val Leu Glu Leu Asp Asn Cys Leu  
 340 345 350  
 Leu Ile Thr Asp Val Ala Leu Glu His Leu Glu Asn Cys Arg Gly Leu  
 355 360 365  
 Glu Arg Leu Glu Leu Tyr Asp Cys Gln Gln Val Thr Arg Ala Gly Ile  
 370 375 380  
 Lys Arg Met Arg Ala Gln Leu Pro His Val Lys Val His Ala Tyr Phe  
 385 390 395 400  
 Ala Pro Val Thr Pro Pro Thr Ala Val Ala Gly Ser Gly Gln Arg Leu  
 405 410 415  
 Cys Arg Cys Cys Val Ile Leu Glx Gln Gln Leu Pro Gly Pro Lys Gly  
 420 425 430  
 Glx Glx Gly Ile Leu Ser Ser Arg Arg Pro Glu Ser Ser Glx Pro Thr  
 435 440 445  
 Pro Pro Ser Pro Asn Leu Leu Ile Leu His Trp Glu Arg His Leu Gln  
 450 455 460  
 Val Lys  
 465

<210> 28  
 <211> 2797  
 <212> DNA  
 <213> Homo sapiens

<400> 28  
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 ctgggtggggc tctactgcga caagctttct aaaaccaatt tttccaacaa caacgatttc 120  
 cgtgctcttc tgcaagtctt gtatgctact ttcaaggagt tcaaaatgca tgagcagatt 180  
 gaaaatgaat acattattgg tttgcttcaa caacgcagcc agaccattta taatgtacat 240  
 tctgacaata aactctccga gatgcttagc ctctttgaaa agggactgaa gaatgttaag 300  
 aatgaatatg aacagttaaa ttatgcaaaa caactgaaag agagattgga ggctttttaca 360  
 agagattttc ttcctcacat gaaagaggaa gaggagggtt ttcagcccat gttaatggaa 420  
 tattttacct atgaagagct taaggatatt aaaaagaaa tgattgcaca aactgctct 480  
 cagaaggata ctgcagaact ccttagaggt cttagcctat ggaatcatgc tgaagagcga 540  
 cagaaatttt ttaaatattc cgtggatgaa aagtcagata aagaagcaga agtgtcagaa 600  
 cactccacag gtataaccca tcttcctcct gaggtaatgc tgtcaatttt cagctatctt 660  
 aatcctcaag agttatgtcg atgcagtcaa gtaagcatga aatgggtctca gctgacaaaa 720  
 acgggatcgc tttggaaaca tctttaccct gttcattggg ccagaggtga ctggtatagt 780  
 ggtcccgcga ctgaacttga tactgaacct gatgatgaat ggggtgaaaa taggaaagat 840  
 gaaagtcgtg cttttcatga gtgggatgaa gatgctgaca ttgatgaatc tgaagagtct 900  
 gcggaggaaat caattgctat cagcattgca caaatggaaa aacgtttact ccatggctta 960  
 attcataacg ttctaccata tgttggtact tctgtaaaaa ccttagtatt agcatcacgc 1020  
 tctgcagttt ccagcaaaaat ggtaggcag atttttagagc tttgtcctaa cctggagcat 1080  
 ctggatctta cccagactga catttcagat tctgcatttg acagttgggt ttggcttgggt 1140  
 tgctgccaga gtcttcggca tcttgatctg tctgggtgtg agaaaatcac agatgtggcc 1200  
 ctagagaaga tttccagagc tcttggaatt ctgacatctc atcaaagtgg ctttttgaaa 1260

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acatctacaa gcaaaattac ttcaactgcg tggaaaaata aagacattac catgcagtcc 1320
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aatgaacacc cctggactaa gcctgtttct tctgagaatt tcacttctcc ttatgtgtgg 1440
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gttgaaaagtc tttgtgtaat ggaaacagca tccaacttta gttgttccac ctctggttgt 1560
tttagtaagg acattgttgg actaaggact agtgtctgtt ggcagcagca ttgtgcttct 1620
ccagcctttg cgtattgtgg tcaactcattt tgtgtacag gaacagcttt aagaactatg 1680
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ctgtttctca gtttatctgg atgttatcag atcacagacc atgggtctcag ggttttgact 1860
ctgggaggag ggctgcctta tttggagcac cttaatctct ctggttgtct tactataact 1920
ggtgcaggcc tgcaggattt ggtttcagca tgtccttctc tgaatgatga atacttttac 1980
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aaccatccct ttttgagcgt gacttgtttt ggccccattt cttacaactt cagaaatctt 2220
aatttaccag tgaattgtaa tgttgtttct cttgcaaatt atacttttgg tttagaaaagg 2280
gattaggtct tttcaaaagg gtgagaacag tcttacattt ttcttttaaa tgaaatgctt 2340
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tttccgacac acatgtctga agacttattt tcaaagacag cacatttttg gaaactaatc 2520
tcttttccgt aatatttctt ttatttcaat gattctcaga aggccaatc aaacaaaccc 2580
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aggaaagcac caatcgatat ttgtttcctt tagggatact ttgttctcac cactgtccct 2700
atgtcatcaa atttgggaga gattttttaa aataccaaa tcatttgaag aaatgtataa 2760
ataaaatcta ctttgaggac tttaccaagt aatatat 2797

```

&lt;210&gt; 29

&lt;211&gt; 691

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 29

```

Met Ala Pro Phe Pro Glu Glu Val Asp Val Phe Thr Ala Pro His Trp
  1              5              10              15

```

```

Arg Met Lys Gln Leu Val Gly Leu Tyr Cys Asp Lys Leu Ser Lys Thr
          20              25              30

```

```

Asn Phe Ser Asn Asn Asn Asp Phe Arg Ala Leu Leu Gln Ser Leu Tyr
          35              40              45

```

```

Ala Thr Phe Lys Glu Phe Lys Met His Glu Gln Ile Glu Asn Glu Tyr
          50              55              60

```

```

Ile Ile Gly Leu Leu Gln Gln Arg Ser Gln Thr Ile Tyr Asn Val His
          65              70              75              80

```

```

Ser Asp Asn Lys Leu Ser Glu Met Leu Ser Leu Phe Glu Lys Gly Leu
          85              90              95

```

```

Lys Asn Val Lys Asn Glu Tyr Glu Gln Leu Asn Tyr Ala Lys Gln Leu
          100             105             110

```

```

Lys Glu Arg Leu Glu Ala Phe Thr Arg Asp Phe Leu Pro His Met Lys
          115             120             125

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Glu Glu Glu Glu Val Phe Gln Pro Met Leu Met Glu Tyr Phe Thr Tyr  
 130 135 140  
 Glu Glu Leu Lys Asp Ile Lys Lys Lys Val Ile Ala Gln His Cys Ser  
 145 150 155 160  
 Gln Lys Asp Thr Ala Glu Leu Leu Arg Gly Leu Ser Leu Trp Asn His  
 165 170 175  
 Ala Glu Glu Arg Gln Lys Phe Phe Lys Tyr Ser Val Asp Glu Lys Ser  
 180 185 190  
 Asp Lys Glu Ala Glu Val Ser Glu His Ser Thr Gly Ile Thr His Leu  
 195 200 205  
 Pro Pro Glu Val Met Leu Ser Ile Phe Ser Tyr Leu Asn Pro Gln Glu  
 210 215 220  
 Leu Cys Arg Cys Ser Gln Val Ser Met Lys Trp Ser Gln Leu Thr Lys  
 225 230 235 240  
 Thr Gly Ser Leu Trp Lys His Leu Tyr Pro Val His Trp Ala Arg Gly  
 245 250 255  
 Asp Trp Tyr Ser Gly Pro Ala Thr Glu Leu Asp Thr Glu Pro Asp Asp  
 260 265 270  
 Glu Trp Val Lys Asn Arg Lys Asp Glu Ser Arg Ala Phe His Glu Trp  
 275 280 285  
 Asp Glu Asp Ala Asp Ile Asp Glu Ser Glu Glu Ser Ala Glu Glu Ser  
 290 295 300  
 Ile Ala Ile Ser Ile Ala Gln Met Glu Lys Arg Leu Leu His Gly Leu  
 305 310 315 320  
 Ile His Asn Val Leu Pro Tyr Val Gly Thr Ser Val Lys Thr Leu Val  
 325 330 335  
 Leu Ala Tyr Ser Ser Ala Val Ser Ser Lys Met Val Arg Gln Ile Leu  
 340 345 350  
 Glu Leu Cys Pro Asn Leu Glu His Leu Asp Leu Thr Gln Thr Asp Ile  
 355 360 365  
 Ser Asp Ser Ala Phe Asp Ser Trp Ser Trp Leu Gly Cys Cys Gln Ser  
 370 375 380  
 Leu Arg His Leu Asp Leu Ser Gly Cys Glu Lys Ile Thr Asp Val Ala  
 385 390 395 400  
 Leu Glu Lys Ile Ser Arg Ala Leu Gly Ile Leu Thr Ser His Gln Ser  
 405 410 415  
 Gly Phe Leu Lys Thr Ser Thr Ser Lys Ile Thr Ser Thr Ala Trp Lys  
 420 425 430  
 Asn Lys Asp Ile Thr Met Gln Ser Thr Lys Gln Tyr Ala Cys Leu His

435	440	445
Asp Leu Thr Asn Lys Gly Ile Gly Glu Glu Ile Asp Asn Glu His Pro 450 455 460		
Trp Thr Lys Pro Val Ser Ser Glu Asn Phe Thr Ser Pro Tyr Val Trp 465 470 475 480		
Met Leu Asp Ala Glu Asp Leu Ala Asp Ile Glu Asp Thr Val Glu Trp 485 490 495		
Arg His Arg Asn Val Glu Ser Leu Cys Val Met Glu Thr Ala Ser Asn 500 505 510		
Phe Ser Cys Ser Thr Ser Gly Cys Phe Ser Lys Asp Ile Val Gly Leu 515 520 525		
Arg Thr Ser Val Cys Trp Gln Gln His Cys Ala Ser Pro Ala Phe Ala 530 535 540		
Tyr Cys Gly His Ser Phe Cys Cys Thr Gly Thr Ala Leu Arg Thr Met 545 550 555 560		
Ser Ser Leu Pro Glu Ser Ser Ala Met Cys Arg Lys Ala Ala Arg Thr 565 570 575		
Arg Leu Pro Arg Gly Lys Asp Leu Ile Tyr Phe Gly Ser Glu Lys Ser 580 585 590		
Asp Gln Glu Thr Gly Arg Val Leu Leu Phe Leu Ser Leu Ser Gly Cys 595 600 605		
Tyr Gln Ile Thr Asp His Gly Leu Arg Val Leu Thr Leu Gly Gly Gly 610 615 620		
Leu Pro Tyr Leu Glu His Leu Asn Leu Ser Gly Cys Leu Thr Ile Thr 625 630 635 640		
Gly Ala Gly Leu Gln Asp Leu Val Ser Ala Cys Pro Ser Leu Asn Asp 645 650 655		
Glu Tyr Phe Tyr Tyr Cys Asp Asn Ile Asn Gly Pro His Ala Asp Thr 660 665 670		
Ala Ser Gly Cys Gln Asn Leu Gln Cys Gly Phe Arg Ala Cys Cys Arg 675 680 685		
Ser Gly Glu 690		

&lt;210&gt; 30

&lt;211&gt; 666

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 30

gtgcagcaac agcagcagca gccccgcag cagccgccgc cgcagccgcc ccagcagcag 60

```

ccgccccagc agcagcctcc gccgcccgcc cagcagcagc agcagcagca gcctccgccg 120
ccgccaccgc cgcctccgcc gctgcctcag gagcggaaca acgtcggcga gcgggatgat 180
gatgtgcctg cagatatggt tgcagaagaa tcaggctctg gtgcacaaaa tagtccatac 240
caacttcgta gaaaaactct tttgccgaaa agaacagcgt gtcccacaaa gaacagtatg 300
gagggcgcct caacttcaac tacagaaaac tttggtcatc gtgcaaaacg tgcaagagtg 360
tctggaaaat cacaagatct atcagcagca cctgctgaac agtatcttca ggagaaaactg 420
ccagatgaag tggttctaaa aatcttctct tacttgctgg aacaggatct ttgtagagca 480
gcttgtgtat gtaaacgctt cagtgaactt gctaattgat caattttgtg gctaggcgaa 540
gtggctcacg cctataatcc cagcactttg ggaggctgaa gtgggcagat tacttgagcc 600
caggagtttg agaccagcct gggcaacata ctgggacctc atttctacaa aaaaaaaaaa 660
aaaaaa

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&lt;210&gt; 31

&lt;211&gt; 192

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 31

```

Val Gln Gln Gln Gln Gln Pro Pro Gln Gln Pro Pro Pro Gln Pro
  1              5              10              15

Pro Gln Gln Gln Pro Pro Gln Gln Gln Pro Pro Pro Pro Pro Gln Gln
      20              25              30

Gln Gln Gln Gln Gln Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro Leu
      35              40              45

Pro Gln Glu Arg Asn Asn Val Gly Glu Arg Asp Asp Asp Val Pro Ala
      50              55              60

Asp Met Val Ala Glu Glu Ser Gly Pro Gly Ala Gln Asn Ser Pro Tyr
      65              70              75              80

Gln Leu Arg Arg Lys Thr Leu Leu Pro Lys Arg Thr Ala Cys Pro Thr
      85              90              95

Lys Asn Ser Met Glu Gly Ala Ser Thr Ser Thr Thr Glu Asn Phe Gly
      100             105             110

His Arg Ala Lys Arg Ala Arg Val Ser Gly Lys Ser Gln Asp Leu Ser
      115             120             125

Ala Ala Pro Ala Glu Gln Tyr Leu Gln Glu Lys Leu Pro Asp Glu Val
      130             135             140

Val Leu Lys Ile Phe Ser Tyr Leu Leu Glu Gln Asp Leu Cys Arg Ala
      145             150             155             160

Ala Cys Val Cys Lys Arg Phe Ser Glu Leu Ala Asn Asp Pro Ile Leu
      165             170             175

Trp Leu Gly Glu Val Ala His Ala Tyr Asn Pro Ser Thr Leu Gly Gly
      180             185             190

```



<210> 32  
 <211> 1256  
 <212> DNA  
 <213> Mus musculus

<400> 32  
 tgaaaggagt caaagaaagc tgccttagtg caacgatagc aaccccagaa ccagaggctt 60  
 gaagaaggaa gccagctgct gaggtgttgt ctttcttatg gaaaaatacc agttcatttc 120  
 atgatggcct ttgcacctcc aaaaagcatc gatggcccca aaatgcagac aaagatgagt 180  
 acctggacac ctctcaacca tcagcttctg aatgaccagg tatttgaaga acgaagagct 240  
 ctgctgggaa aatggtttga caaatggaca gactctcaaa ggagaagaat tcttacaggc 300  
 ctactagagc gctgttcctt gtcccagcag aagttctgtt gtcgaaagct gcaggaaaaa 360  
 atcccagcag aggccctgga ttttactacc aagcttccaa ggggtgttatc tgtctacatc 420  
 ttttcttccc tggatccccg gagtctttgc cgttgtgcac aggtgagctg gtactggaag 480  
 agcttggctg agttggacca gctctggatg ctcaagtgcc tgcgcttcaa ctggtacatc 540  
 agcttctccc caacgcctt tgaacagggc gtctggaaaa agcactacat ccagatgggtg 600  
 agagaacttc acgtcaccaa gcccaagaca cctccaaagg atgaattcac aactgccgat 660  
 gttcagccaa ttcttgga tttctccagat gagaagcagt ccccttctct agcttttcgg 720  
 tctctctcct ccttaagaaa gaagaataac cccggggaga aagagcttcc accttggcga 780  
 tcgtcagata agcatccac tgacatcatc cgctttaatt atctagacaa ctgtgaccct 840  
 gagctcttca ggctaggaag aagaaaaaga agcgaagtga cccagactt caagcgccag 900  
 ctgcgagata agaaaaataa gcttcaagac agagccaggc tcaggaaagc acagtcattg 960  
 atctccctga gtccccctcc aaaagttcca gtctgtctcg cctggcctct gcactctgcca 1020  
 gtggcaccct cagaccgtga agcagcaacg gaagcactcc tggagcatct tcagaaacac 1080  
 cccgggcttc agtcaccctc accgaggctc cagagtcaaa gctgattaaa ggattggaaa 1140  
 aggacttttt ctagaaacga caagggtgtt ttcacagctg agagttggat tctagctagt 1200  
 cctccatta aactcttttg gcttaagtaa aaaaaaaaaa aaaaaaaaaa aaaaaa 1256

<210> 33  
 <211> 373  
 <212> PRT  
 <213> Mus musculus

<400> 33  
 Lys Glu Ser Lys Lys Ala Ala Leu Val Gln Arg Glx Gln Pro Gln Asn  
 1 5 10 15  
 Gln Arg Leu Glu Glu Gly Ser Gln Leu Leu Arg Cys Cys Leu Ser Tyr  
 20 25 30  
 Gly Lys Ile Pro Val His Phe Met Met Ala Phe Ala Pro Pro Lys Ser  
 35 40 45  
 Ile Asp Gly Pro Lys Met Gln Thr Lys Met Ser Thr Trp Thr Pro Leu  
 50 55 60  
 Asn His Gln Leu Leu Asn Asp Gln Val Phe Glu Glu Arg Arg Ala Leu  
 65 70 75 80  
 Leu Gly Lys Trp Phe Asp Lys Trp Thr Asp Ser Gln Arg Arg Arg Ile  
 85 90 95  
 Leu Thr Gly Leu Leu Glu Arg Cys Ser Leu Ser Gln Gln Lys Phe Cys  
 100 105 110  
 Cys Arg Lys Leu Gln Glu Lys Ile Pro Ala Glu Ala Leu Asp Phe Thr  
 115 120 125

Thr Lys Leu Pro Arg Val Leu Ser Val Tyr Ile Phe Ser Phe Leu Asp  
 130 135 140  
 Pro Arg Ser Leu Cys Arg Cys Ala Gln Val Ser Trp Tyr Trp Lys Ser  
 145 150 155 160  
 Leu Ala Glu Leu Asp Gln Leu Trp Met Leu Lys Cys Leu Arg Phe Asn  
 165 170 175  
 Trp Tyr Ile Ser Phe Ser Pro Thr Pro Phe Glu Gln Gly Val Trp Lys  
 180 185 190  
 Lys His Tyr Ile Gln Met Val Arg Glu Leu His Val Thr Lys Pro Lys  
 195 200 205  
 Thr Pro Pro Lys Asp Glu Phe Thr Thr Ala Asp Val Gln Pro Ile Pro  
 210 215 220  
 Gly Asn Ser Pro Asp Glu Lys Gln Ser Pro Ser Leu Ala Phe Arg Ser  
 225 230 235 240  
 Ser Ser Ser Leu Arg Lys Lys Asn Asn Pro Gly Glu Lys Glu Leu Pro  
 245 250 255  
 Pro Trp Arg Ser Ser Asp Lys His Pro Thr Asp Ile Ile Arg Phe Asn  
 260 265 270  
 Tyr Leu Asp Asn Cys Asp Pro Glu Leu Phe Arg Leu Gly Arg Arg Lys  
 275 280 285  
 Arg Ser Glu Val Thr Pro Asp Phe Lys Arg Gln Leu Arg Asp Lys Lys  
 290 295 300  
 Asn Lys Leu Gln Asp Arg Ala Arg Leu Arg Lys Ala Gln Ser Leu Ile  
 305 310 315 320  
 Ser Leu Ser Ser Pro Pro Lys Val Pro Val Arg Leu Ala Trp Pro Leu  
 325 330 335  
 His Leu Pro Val Ala Pro Ser Asp Arg Glu Ala Ala Thr Glu Ala Leu  
 340 345 350  
 Leu Glu His Leu Gln Lys His Pro Gly Leu Gln Ser Pro Ser Pro Arg  
 355 360 365  
 Leu Gln Ser Gln Ser  
 370

&lt;210&gt; 34

&lt;211&gt; 1825

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 34

tggctgtgga ggggacccgg ccgctgacgac gctctggcgg cccgagcgcg cctagtcggt 60  
 gtgagcccg ggcgaggtcc cgggccccgg ggcgctcgct caggtaaata tttccataac 120  
 cttatggaga gaaaggactt tgagacatgg cttgataaca tttctgttac atttctttct 180

```

ctgatggact tgcagaaaaa tgaaactctg gaccacctga ttagtctgag tggggcagtc 240
cagctcaggc atctctccaa taacctggag actctcctca agcgggactt cctcaaactc 300
cttcccctgg agctcagttt ttatttggtt aaatggctcg atcctcagac tttactcaca 360
tgctgcctgg tctctaagca gcggaataag gtgataagtg cctgtacaga ggtgtggcag 420
actgcatgta aaaatttggg ctggcagata gatgattctg ttcaggactc attgcaactg 480
aagaaggttt atttgaaggc tattttgagg atgaagcaac tggaggacca tgaagccttt 540
gagacctctt cgtaaattgg acatagtgcc agagtgtatg cactttacta caaagatgga 600
cttctctgta cagggtcaga tgacttgtct gcaaagctgt gggatgtaag cacagggcag 660
tgtgtttacg gcatccagac ccacacttgt gcagctgtga agttcgatga acagaagctt 720
gtgacaggct cctttgacaa cactgtggcc tgctgggagt ggagttccgg agccaggacc 780
cagcacttcc gggggcacac gggggcggtg ttcagtgtgg actacagtga tgaactggat 840
attttggtga gtggctctgc ggacttcgct gtgaaagtat gggctttatc tgctgggaca 900
tgctgaata cactcactgg gcatactgaa tgggtcacca aggtgggttt gcagaagtgc 960
aaagtcaagt ctctcttgca cagccctgga gactacatcc tcttaagtgc agacaaatat 1020
gagatcaaga tttggccaat tgggagagaa atcaactgta agtgcttgaa gacactgtct 1080
gtctctgagg atagaagtat ctgcctgcag ccaagacttc attttgatgg aaaatacatt 1140
gtctgtagtt cagccctggg tctgtaccag tgggactttg ccagttatga tattctcagg 1200
gtcatcaaga cacctgaggt agcaaacttg gccttgcttg gctttggaga tgtcttcgcc 1260
ctgctgtttg acaaccacta cctatatatc atggacttga ggacagagag cctaattagc 1320
cgctggcctc tgccagagta caggaaatca aagagaggca ccagcttcct ggcaggcgaa 1380
cgctctggtt gaatggattg gatgggcaca atgacacggg cttagtcttt gccaccagca 1440
tgcttgacca cagtattcac ctggtgttat ggaaggagca ttgctgacac caggagctac 1500
caccgctgac tgactttggg tgccagggtc gcggtttttg ggtgcaatgt ctatggcagc 1560
caactgcatg aaccaaaagt ctcacctaaa ggtatcatca cgcagtgcac aatcatgtat 1620
ctgtttgcca gggctggggc ggggagggtc tgtttactga catacaccgc agcatgctaa 1680
tgggatacac cattgacttc atttgatctt agttatgttg gtcagtgtaa gagaggttgc 1740
atttttggat ttatctttct gagtgaata ttgagtaaag aaagttaa at gattcactaa 1800
tctgccta at tgggtgccca taaaa 1825

```

&lt;210&gt; 35

&lt;211&gt; 422

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 35

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Met Glu Arg Lys Asp Phe Glu Thr Trp Leu Asp Asn Ile Ser Val Thr
  1                      5                      10                      15

```

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Phe Leu Ser Leu Met Asp Leu Gln Lys Asn Glu Thr Leu Asp His Leu
      20                      25                      30

```

```

Ile Ser Leu Ser Gly Ala Val Gln Leu Arg His Leu Ser Asn Asn Leu
      35                      40                      45

```

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Glu Thr Leu Leu Lys Arg Asp Phe Leu Lys Leu Leu Pro Leu Glu Leu
      50                      55                      60

```

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Ser Phe Tyr Leu Leu Lys Trp Leu Asp Pro Gln Thr Leu Leu Thr Cys
      65                      70                      75                      80

```

```

Cys Leu Val Ser Lys Gln Arg Asn Lys Val Ile Ser Ala Cys Thr Glu
      85                      90                      95

```

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Val Trp Gln Thr Ala Cys Lys Asn Leu Gly Trp Gln Ile Asp Asp Ser
      100                      105                      110

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Val Gln Asp Ser Leu His Trp Lys Lys Val Tyr Leu Lys Ala Ile Leu

```

115	120	125
Arg Met Lys Gln Leu Glu Asp His Glu Ala Phe Glu Thr Ser Ser Leu 130	135	140
Ile Gly His Ser Ala Arg Val Tyr Ala Leu Tyr Tyr Lys Asp Gly Leu 145	150	155
Leu Cys Thr Gly Ser Asp Asp Leu Ser Ala Lys Leu Trp Asp Val Ser 165	170	175
Thr Gly Gln Cys Val Tyr Gly Ile Gln Thr His Thr Cys Ala Ala Val 180	185	190
Lys Phe Asp Glu Gln Lys Leu Val Thr Gly Ser Phe Asp Asn Thr Val 195	200	205
Ala Cys Trp Glu Trp Ser Ser Gly Ala Arg Thr Gln His Phe Arg Gly 210	215	220
His Thr Gly Ala Val Phe Ser Val Asp Tyr Ser Asp Glu Leu Asp Ile 225	230	235
Leu Val Ser Gly Ser Ala Asp Phe Ala Val Lys Val Trp Ala Leu Ser 245	250	255
Ala Gly Thr Cys Leu Asn Thr Leu Thr Gly His Thr Glu Trp Val Thr 260	265	270
Lys Val Val Leu Gln Lys Cys Lys Val Lys Ser Leu Leu His Ser Pro 275	280	285
Gly Asp Tyr Ile Leu Leu Ser Ala Asp Lys Tyr Glu Ile Lys Ile Trp 290	295	300
Pro Ile Gly Arg Glu Ile Asn Cys Lys Cys Leu Lys Thr Leu Ser Val 305	310	315
Ser Glu Asp Arg Ser Ile Cys Leu Gln Pro Arg Leu His Phe Asp Gly 325	330	335
Lys Tyr Ile Val Cys Ser Ser Ala Leu Gly Leu Tyr Gln Trp Asp Phe 340	345	350
Ala Ser Tyr Asp Ile Leu Arg Val Ile Lys Thr Pro Glu Val Ala Asn 355	360	365
Leu Ala Leu Leu Gly Phe Gly Asp Val Phe Ala Leu Leu Phe Asp Asn 370	375	380
His Tyr Leu Tyr Ile Met Asp Leu Arg Thr Glu Ser Leu Ile Ser Arg 385	390	395
Trp Pro Leu Pro Glu Tyr Arg Lys Ser Lys Arg Gly Thr Ser Phe Leu 405	410	415
Ala Gly Glu Arg Pro Gly 420		

<210> 36  
 <211> 1847  
 <212> DNA  
 <213> Homo sapiens

<400> 36  
 ggactgtctc gtggcaccgc gtggaaccga ggagaacgtg gagcgccggg agcggcgaat 60  
 atggacgact acagcctgga tgagttccgt cggcgctggc aggaggagct ggcgcaggcc 120  
 caggcgccga agaagcggcg acggccccgag gctgccgaga ggcgggctcg gcggccggag 180  
 aatgaaatga atgatgtgcc tttctttgat atccaactgc cttacgaatt ggcaatcaat 240  
 atatttcagt atctggacag gaaagaacta ggaagatgtg cacagggtgag caagacgtgg 300  
 aaggtgattg cagaggatga ggtgctgtgg tacaggctgt gccagcagga agggcacctt 360  
 ccggatagca gcatctctga ctattcttgc tggaaactca tcttccaaga gtgccgagcc 420  
 aaggaacaca tgttacaaac caactggaag aatcgaaaag gtgccgtgag cgagctggag 480  
 catgttcctg acacagtttt gtgtgatgtg cattctcacg atggtgtggt cattgcggga 540  
 tatacatcag gggatgtgag agtgtgggac acccgacct gggactacgt agcccccttc 600  
 ctggaatcag aggacgagga ggatgagcct ggaatgcagc caaatgtctc ctttgtgagg 660  
 ataaacagct cgttggcagt agcagcttat gaggatgggt tcttaataatt tgggattaag 720  
 gaccggaaag taccctgttc atcgtttgag cacgatgcaa gaatacaggc actagccctc 780  
 agccaggacg atgcaaccgt ggccacagct tctgcttttg atgtcgtgat gttatcccc 840  
 aatgaggagg ggtactggca gatagctgcg gaatttgaag ttccgaaaact ggttcagtac 900  
 cttgaaatag ttccagaaac cagaaggtag cctgtggcag tagccgctgc tggagatctg 960  
 atgtacctgc tcaaagccga agactccgcc agaaccctcc tttacgcca cggcccgcc 1020  
 gtcacatgtc tagacgtctc ggccaaccaa gttgcttttg gtgtacaggg tctgggatgg 1080  
 gtgtacgaag gaagcaagat cctggtgtat agcctggaag caggacgccg cctcttgaag 1140  
 ctgggtaacg ttctccgtga cttcacgtgt gtcaacctca gcgacagccc tcccaacctc 1200  
 atggtcagtg gcaacatgga cgggaggggtg aggatccacg acctccgcag tggtaacatc 1260  
 gccctgtcgc tctccgcca tcagctcagg gtctctgctg tgcagatgga tgactggaag 1320  
 atcgtcagtg gaggcgagga aggcctgggtg tccgtgtggg attatcggtat gaaccagaag 1380  
 ctgtggaagg tgtattccgg gcacccgggtg cagcacatct cattcagcag ccacagcctc 1440  
 atcacggcca acgtgcctta ccagacggta atgcgaaaac ccgacctgga cagcttcact 1500  
 actcacagga gacaccgggg gctgatccgc gcctacgagt ttgcggtgga ccagctggcc 1560  
 ttccagagcc ctctccctgt ctgccgttca tctgtgacg ccatggccac tcaactactac 1620  
 gacctcgcac tggcctttcc ctataaccat gtttagggat gtgcctcagt tgggagcaag 1680  
 gagaaaaatg ggaagaacca gttttatcca tcttaaaacg ccaggcacct cttcacagg 1740  
 ggtaaacatt taggggaaga aagcagccca ggggtgccatg cctgacagca cgcattctcc 1800  
 tgaccctgc acttccccca gcgcctgggg caagctggcg tgtgcca 1847

<210> 37  
 <211> 531  
 <212> PRT  
 <213> Homo sapiens

<400> 37  
 Met Asp Asp Tyr Ser Leu Asp Glu Phe Arg Arg Arg Trp Gln Glu Glu  
 1 5 10 15  
 Leu Ala Gln Ala Gln Ala Pro Lys Lys Arg Arg Arg Pro Glu Ala Ala  
 20 25 30  
 Glu Arg Arg Ala Arg Arg Pro Glu Asn Glu Met Asn Asp Val Pro Phe  
 35 40 45  
 Phe Asp Ile Gln Leu Pro Tyr Glu Leu Ala Ile Asn Ile Phe Gln Tyr  
 50 55 60

Leu Asp Arg Lys Glu Leu Gly Arg Cys Ala Gln Val Ser Lys Thr Trp  
 65 70 75 80  
 Lys Val Ile Ala Glu Asp Glu Val Leu Trp Tyr Arg Leu Cys Gln Gln  
 85 90 95  
 Glu Gly His Leu Pro Asp Ser Ser Ile Ser Asp Tyr Ser Cys Trp Lys  
 100 105 110  
 Leu Ile Phe Gln Glu Cys Arg Ala Lys Glu His Met Leu Gln Thr Asn  
 115 120 125  
 Trp Lys Asn Arg Lys Gly Ala Val Ser Glu Leu Glu His Val Pro Asp  
 130 135 140  
 Thr Val Leu Cys Asp Val His Ser His Asp Gly Val Val Ile Ala Gly  
 145 150 155 160  
 Tyr Thr Ser Gly Asp Val Arg Val Trp Asp Thr Arg Thr Trp Asp Tyr  
 165 170 175  
 Val Ala Pro Phe Leu Glu Ser Glu Asp Glu Glu Asp Glu Pro Gly Met  
 180 185 190  
 Gln Pro Asn Val Ser Phe Val Arg Ile Asn Ser Ser Leu Ala Val Ala  
 195 200 205  
 Ala Tyr Glu Asp Gly Phe Leu Ile Phe Gly Ile Lys Asp Arg Lys Val  
 210 215 220  
 Pro Cys Ser Ser Phe Glu His Asp Ala Arg Ile Gln Ala Leu Ala Leu  
 225 230 235 240  
 Ser Gln Asp Asp Ala Thr Val Ala Thr Ala Ser Ala Phe Asp Val Val  
 245 250 255  
 Met Leu Ser Pro Asn Glu Glu Gly Tyr Trp Gln Ile Ala Ala Glu Phe  
 260 265 270  
 Glu Val Pro Lys Leu Val Gln Tyr Leu Glu Ile Val Pro Glu Thr Arg  
 275 280 285  
 Arg Tyr Pro Val Ala Val Ala Ala Ala Gly Asp Leu Met Tyr Leu Leu  
 290 295 300  
 Lys Ala Glu Asp Ser Ala Arg Thr Leu Leu Tyr Ala His Gly Pro Pro  
 305 310 315 320  
 Val Thr Cys Leu Asp Val Ser Ala Asn Gln Val Ala Phe Gly Val Gln  
 325 330 335  
 Gly Leu Gly Trp Val Tyr Glu Gly Ser Lys Ile Leu Val Tyr Ser Leu  
 340 345 350  
 Glu Ala Gly Arg Arg Leu Leu Lys Leu Gly Asn Val Leu Arg Asp Phe  
 355 360 365  
 Thr Cys Val Asn Leu Ser Asp Ser Pro Pro Asn Leu Met Val Ser Gly

370                                      375                                      380  
 Asn Met Asp Gly Arg Val Arg Ile His Asp Leu Arg Ser Gly Asn Ile  
 385                                      390                                      395                                      400  
 Ala Leu Ser Leu Ser Ala His Gln Leu Arg Val Ser Ala Val Gln Met  
                                     405                                      410                                      415  
 Asp Asp Trp Lys Ile Val Ser Gly Gly Glu Glu Gly Leu Val Ser Val  
                                     420                                      425                                      430  
 Trp Asp Tyr Arg Met Asn Gln Lys Leu Trp Lys Val Tyr Ser Gly His  
                                     435                                      440                                      445  
 Pro Val Gln His Ile Ser Phe Ser Ser His Ser Leu Ile Thr Ala Asn  
                                     450                                      455                                      460  
 Val Pro Tyr Gln Thr Val Met Arg Asn Ala Asp Leu Asp Ser Phe Thr  
 465                                      470                                      475                                      480  
 Thr His Arg Arg His Arg Gly Leu Ile Arg Ala Tyr Glu Phe Ala Val  
                                     485                                      490                                      495  
 Asp Gln Leu Ala Phe Gln Ser Pro Leu Pro Val Cys Arg Ser Ser Cys  
                                     500                                      505                                      510  
 Asp Ala Met Ala Thr His Tyr Tyr Asp Leu Ala Leu Ala Phe Pro Tyr  
                                     515                                      520                                      525  
 Asn His Val  
                                     530

<210> 38  
 <211> 1684  
 <212> DNA  
 <213> Homo sapiens

<400> 38  
 ggaagcgagc cgcgcgagcgg aacaaactcg ccgccgccgc ccttcagcga ctggggccgc 60  
 ctggaggcgg ccacccctcag cggctggaag accttctggc agtcagtga caaggagagg 120  
 gtggcgcgta cgacctcacg ggaggagggt gatgaggcgg ccagcaccct gacgcggctg 180  
 ccgattgatg tacagctata tattttgtcc tttctttcac ctcatgatct gtgtcagttg 240  
 ggaagtacaa atcattattg gaatgaaact gtaagagatc caattctgtg gagatacttt 300  
 ttggtgaggg atcttccttc ttggtcttct gttgactgga agtctcttcc agatctagaa 360  
 atcttaaaaa agcctatatc tgagggtcact gatggtgcat tttttgacta catggcagtc 420  
 tatagaatgt gctgtccata cacaagaaga gcttcaaaat ccagccgtcc tatgtatgga 480  
 gctgtcactt ctttttttaca ctccctgatc attcagaatg aaccacgatt tgctatgttt 540  
 ggaccagggt tggaagaatt gaatacctct ttggtgttga gcttgatgtc ttcagaggaa 600  
 ctttgcccaa cagctgggtt gcctcagagg cagattgatg gtattggatc aggagtcaat 660  
 tttcagttga acaaccaaca taaattcaac attctaattc tatattcaac taccagaaag 720  
 gaaagagata gagcaaggga agagcatata agtgcagtta acaagatgtt cagtcgacac 780  
 aatgaagggt atgatcaaca aggaagccgg tacagtgtga ttccacagat tcaaaaagtg 840  
 tgtgaagttg tagatgggtt catctatgtt gcaaagtctg aagctcataa aagtaagtac 900  
 tcatatgtac atttttaagc acattgttct tttcaaagca aatggaaaat acctttttag 960  
 actttactgt ggcttcttaa gaatatgatg gcttatttta tcaatgtgat tttctgacct 1020  
 ttactattaa tagtgtgttc tcacactttt gtgtgtatta ggatcacctg ggaagcttgt 1080  
 taaacatggt aacaagctga ttcctaccct ggtggatttt attcctagat ttgggttgtg 1140

```

tgaatcagat ttgggcatct gttttttcca agcaccagg gaattctgat gcattccaaa 1200
gtttggtact agagctatac aatatttttt tgctttatgc ttctgcatct ttttgatact 1260
gacattttgt ctgcttctgg ctttattcaa ataattctatg caaataacta tgaaaaattt 1320
tatctttggt ttatccaaat agtaaagaga tgtgatggta ggggtgtaag tagattagtt 1380
gagaacacta ttcatatatt ctgaataatt gccattcaga atgctatatt tgtgtgaaat 1440
caaaatatca ttggggccatg agctagtatt ataatatctt tcccaaattc tttgtgaaca 1500
aggtagaata taaatacata atatattgta tatatattat agttctctaa tacactgggt 1560
tgaattttcta ttcaatttat tttccctgt gatctgtag gcatgaaagc agttgcaatt 1620
actttctggt attacagggc tatctataaa aagaaaactt agaagcaaaa aaaaaaaaaa 1680
aaaa

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<210> 39  
 <211> 305  
 <212> PRT  
 <213> Homo sapiens

```

<400> 39
Gly Ser Glu Pro Arg Ser Gly Thr Asn Ser Pro Pro Pro Phe Ser
  1              5              10              15

Asp Trp Gly Arg Leu Glu Ala Ala Ile Leu Ser Gly Trp Lys Thr Phe
      20              25              30

Trp Gln Ser Val Ser Lys Glu Arg Val Ala Arg Thr Thr Ser Arg Glu
      35              40              45

Glu Val Asp Glu Ala Ala Ser Thr Leu Thr Arg Leu Pro Ile Asp Val
      50              55              60

Gln Leu Tyr Ile Leu Ser Phe Leu Ser Pro His Asp Leu Cys Gln Leu
      65              70              75              80

Gly Ser Thr Asn His Tyr Trp Asn Glu Thr Val Arg Asp Pro Ile Leu
      85              90              95

Trp Arg Tyr Phe Leu Leu Arg Asp Leu Pro Ser Trp Ser Ser Val Asp
      100             105             110

Trp Lys Ser Leu Pro Asp Leu Glu Ile Leu Lys Lys Pro Ile Ser Glu
      115             120             125

Val Thr Asp Gly Ala Phe Phe Asp Tyr Met Ala Val Tyr Arg Met Cys
      130             135             140

Cys Pro Tyr Thr Arg Arg Ala Ser Lys Ser Ser Arg Pro Met Tyr Gly
      145             150             155             160

Ala Val Thr Ser Phe Leu His Ser Leu Ile Ile Gln Asn Glu Pro Arg
      165             170             175

Phe Ala Met Phe Gly Pro Gly Leu Glu Glu Leu Asn Thr Ser Leu Val
      180             185             190

Leu Ser Leu Met Ser Ser Glu Glu Leu Cys Pro Thr Ala Gly Leu Pro
      195             200             205

Gln Arg Gln Ile Asp Gly Ile Gly Ser Gly Val Asn Phe Gln Leu Asn

```



210

215

220

Asn Gln His Lys Phe Asn Ile Leu Ile Leu Tyr Ser Thr Thr Arg Lys  
 225 230 235 240

Glu Arg Asp Arg Ala Arg Glu Glu His Thr Ser Ala Val Asn Lys Met  
 245 250 255

Phe Ser Arg His Asn Glu Gly Asp Asp Gln Gln Gly Ser Arg Tyr Ser  
 260 265 270

Val Ile Pro Gln Ile Gln Lys Val Cys Glu Val Val Asp Gly Phe Ile  
 275 280 285

Tyr Val Ala Asn Ala Glu Ala His Lys Ser Lys Tyr Ser Tyr Val His  
 290 295 300

Phe  
 305

<210> 40  
 <211> 1025  
 <212> DNA  
 <213> Homo sapiens

<400> 40  
 aagtcctccgc ccagaggcca attcgtcgcg gcggcggttg agatcgcagg tcgctcaggc 60  
 ttgcagatgg gtcaagggtt gtggagagtg gtcagaaacc agcagctgca acaagaaggc 120  
 tacagtgagc aaggctacct caccagagag cagagcagga gaatggctgc gagcaacatt 180  
 tctaacacca atcatcgtaa acaagtccaa ggaggcattg acatataatca tcttttgaag 240  
 gcaaggaaat cgaaagaaca ggaaggattc attaatattg aaatgttgcc tcctgagcta 300  
 agctttacca tcttgtccta cctgaatgca actgaccttt gcttggttc atgtgtttg 360  
 caggaccttg cgaatgatga acttctcttg caagggttg gcaaattcac ttgggggtcac 420  
 tggtccatat acaataagaa cccaccttta ggattttctt ttagaaaatg tatatgcagc 480  
 tggatgaagg cagcctcacc tttaatgcca acccagatga gggagtgaac tactttatgt 540  
 ccaagggtat cctggatgat tcgccaaagg aaatagcaaaa gtttatcttc tgtacaagaa 600  
 cactaaattg gaaaaaactg agaattctatc ttgatgaaag gagagatgct ttggatgacc 660  
 ttgtaacatt gcataatttt agaaatcagt tcttgccaaa tgcactgaga gaattttttc 720  
 gtcatatcca tgcccttgaa gagcgtggag agtatcttga aactcttata acaaagttct 780  
 cacatagatt ctgtgcttgc aacctgatt taatgcgaga acttggcctt agtcctgatg 840  
 ctgtctatgt actgtgctac tctttgattc tactttccat tgacctcact agccctcatg 900  
 tgaagaataa aatgtcaaaa aggaatttta ttcgaaatac ccgtcgcgct gctcaaaaata 960  
 ttagtgaaga ttttgtaggg catctttatg acaatatcta ccttattggc catgtggctg 1020  
 cataa 1025

<210> 41  
 <211> 175  
 <212> PRT  
 <213> Homo sapiens

<400> 41  
 Lys Ser Pro Pro Arg Gly Gln Phe Val Ala Ala Val Glu Ile Ala  
 1 5 10 15

Gly Arg Ser Gly Leu Gln Met Gly Gln Gly Leu Trp Arg Val Val Arg  
 20 25 30

46

Asn Gln Gln Leu Gln Gln Glu Gly Tyr Ser Glu Gln Gly Tyr Leu Thr  
                   35                                  40                                  45  
 Arg Glu Gln Ser Arg Arg Met Ala Ala Ser Asn Ile Ser Asn Thr Asn  
                   50                                  55                                  60  
 His Arg Lys Gln Val Gln Gly Gly Ile Asp Ile Tyr His Leu Leu Lys  
                   65                                  70                                  75                                  80  
 Ala Arg Lys Ser Lys Glu Gln Glu Gly Phe Ile Asn Leu Glu Met Leu  
                                   85                                  90                                  95  
 Pro Pro Glu Leu Ser Phe Thr Ile Leu Ser Tyr Leu Asn Ala Thr Asp  
                   100                                  105                                  110  
 Leu Cys Leu Ala Ser Cys Val Trp Gln Asp Leu Ala Asn Asp Glu Leu  
                   115                                  120                                  125  
 Leu Trp Gln Gly Leu Cys Lys Ser Thr Trp Gly His Cys Ser Ile Tyr  
                   130                                  135                                  140  
 Asn Lys Asn Pro Pro Leu Gly Phe Ser Phe Arg Lys Cys Ile Cys Ser  
                   145                                  150                                  155                                  160  
 Trp Met Lys Ala Ala Ser Pro Leu Met Pro Thr Gln Met Arg Glu  
                                   165                                  170                                  175

&lt;210&gt; 42

&lt;211&gt; 2151

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 42

tgcgttggtgct gcggcctggc accaaagggg cggccccggc ggagagcgga cccagtggcc 60  
 tcggcgatta tggaccggc cgaggcgggt ctgcaagaga aggcactcaa gtttatgaat 120  
 tcctcagaga gagaagactg taataatggc gaaccccta ggaagataat accagagaag 180  
 aattcactta gacagacata caacagctgt gccagactct gcttaaacca agaaacagta 240  
 tgttttagcaa gcactgctat gaagactgag aattgtgtgg ccaaaacaaa acttgccaat 300  
 ggcacttcca gtatgattgt gcccaagcaa cggaaactct cagcaagcta tgaaaaggaa 360  
 aaggaactgt gtgtcaataa ctttgagcag tggtcagagt cagatcaagt ggaatttgtg 420  
 gaacatctta tatcccaaat gtgtcattac caacatgggc acataaactc gtatcttaaa 480  
 cctatgttgc agagagattt cataactgct ctgccagctc ggggattgga tcatatcgct 540  
 gagaacattc tgtcatacct ggatgccaaa tcactatgtg ctgctgaact tgtgtgcaag 600  
 gaatggtacc gagtgcctc tgatggcatg ctgtggaaga agcttatcga gagaatggtc 660  
 aggacagatt ctctgtggag aggcctggca gaacgaagag gatggggaca gtatttatc 720  
 aaaaacaaac ctctgacgg gaatgctcct cccaactctt tttatagagc actttatcct 780  
 aaaattatac aagacattga gacaatagaa tctaattgga gatgtggaag acatagttaa 840  
 cagagaattc actgccgaag tgaaacaagc aaaggagttt actggttaca gtatgatgat 900  
 cagaaaatag taagcggcct tcgagacaac acaatcaaga tctgggataa aaacacattg 960  
 gaatgcaagc gaattctcac aggccatata ggttcagtc tctgtctcca gtatgatgag 1020  
 agagtgatca taacaggatc atcggattcc acggtcagag tgtgggatgt aaatacagggt 1080  
 gaaatgctaa acacgttgat tcaccattgt gaagcagttc tgcacttgcg tttcaataat 1140  
 ggcatgatgg tgacctgctc caaagatcgt tccattgctg tatgggatat ggcctcccca 1200  
 actgacatta ccctccggag ggtgctggtc ggacaccgag ctgctgtcaa tgtttagtag 1260  
 tttgatgaca agtacattgt ttctgcatct ggggatagaa ctataaagggt atggaacaca 1320  
 agtacttgtg aatttgtaag gaccttaaat ggacacaaac gaggcattgc ctgtttgcag 1380  
 tacagggaca ggctggtagt gagtggctca tctgacaaca ctatcagatt atggggacata 1440

```

gaatgtggtg catgtttacg agtggttagaa ggccatgagg aattggtgcg ttgtattcga 1500
tttgataaca agaggatagt cagtggggcc tatgatggaa aaattaaagt gtgggatctt 1560
gtggctgctt tggacccccg tgctcctgca gggacactct gtctacggac ccttgtggag 1620
cattccggaa gagtttttctg actacagttt gatgaattcc agattgtcag tagttcacat 1680
gatgacacaa tcctcatctg ggacttccta aatgatccag ctgcccgaagc tgaaccccc 1740
cgttccccctt ctcgaacata cacctacatc tccagataaa taaccataca ctgacctcat 1800
acttgcccag gaccatttaa agttgcggta tttaacgtat ctgccaatac caggatgagc 1860
aacaacagta acaatcaaac tactgcccag tttccctgga ctagccgagg agcagggtt 1920
tgagactcct gttgggacac agttggtctg cagtcggccc aggacggtct actcagcaca 1980
actgactgct tcagtgtctg tatcagaaga tgtcttctat caattgtgaa tgattggaac 2040
ttttaaacct cccctcctct cctcctttca cctctgcacc tagttttttc ccattgggtc 2100
cagacaaagg tgacttataa atatatttag tgttttgcca gaaaaaaaaa a 2151

```

&lt;210&gt; 43

&lt;211&gt; 569

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 43

```

Met Asp Pro Ala Glu Ala Val Leu Gln Glu Lys Ala Leu Lys Phe Met
  1              5              10              15

```

```

Asn Ser Ser Glu Arg Glu Asp Cys Asn Asn Gly Glu Pro Pro Arg Lys
      20              25              30

```

```

Ile Ile Pro Glu Lys Asn Ser Leu Arg Gln Thr Tyr Asn Ser Cys Ala
    35              40              45

```

```

Arg Leu Cys Leu Asn Gln Glu Thr Val Cys Leu Ala Ser Thr Ala Met
    50              55              60

```

```

Lys Thr Glu Asn Cys Val Ala Lys Thr Lys Leu Ala Asn Gly Thr Ser
    65              70              75              80

```

```

Ser Met Ile Val Pro Lys Gln Arg Lys Leu Ser Ala Ser Tyr Glu Lys
      85              90              95

```

```

Glu Lys Glu Leu Cys Val Lys Tyr Phe Glu Gln Trp Ser Glu Ser Asp
    100              105              110

```

```

Gln Val Glu Phe Val Glu His Leu Ile Ser Gln Met Cys His Tyr Gln
    115              120              125

```

```

His Gly His Ile Asn Ser Tyr Leu Lys Pro Met Leu Gln Arg Asp Phe
    130              135              140

```

```

Ile Thr Ala Leu Pro Ala Arg Gly Leu Asp His Ile Ala Glu Asn Ile
    145              150              155              160

```

```

Leu Ser Tyr Leu Asp Ala Lys Ser Leu Cys Ala Ala Glu Leu Val Cys
      165              170              175

```

```

Lys Glu Trp Tyr Arg Val Thr Ser Asp Gly Met Leu Trp Lys Lys Leu
    180              185              190

```

```

Ile Glu Arg Met Val Arg Thr Asp Ser Leu Trp Arg Gly Leu Ala Glu
    195              200              205

```

48

Arg Arg Gly Trp Gly Gln Tyr Leu Phe Lys Asn Lys Pro Pro Asp Gly  
 210 215 220  
 Asn Ala Pro Pro Asn Ser Phe Tyr Arg Ala Leu Tyr Pro Lys Ile Ile  
 225 230 235 240  
 Gln Asp Ile Glu Thr Ile Glu Ser Asn Trp Arg Cys Gly Arg His Ser  
 245 250 255  
 Leu Gln Arg Ile His Cys Arg Ser Glu Thr Ser Lys Gly Val Tyr Cys  
 260 265 270  
 Leu Gln Tyr Asp Asp Gln Lys Ile Val Ser Gly Leu Arg Asp Asn Thr  
 275 280 285  
 Ile Lys Ile Trp Asp Lys Asn Thr Leu Glu Cys Lys Arg Ile Leu Thr  
 290 295 300  
 Gly His Thr Gly Ser Val Leu Cys Leu Gln Tyr Asp Glu Arg Val Ile  
 305 310 315 320  
 Ile Thr Gly Ser Ser Asp Ser Thr Val Arg Val Trp Asp Val Asn Thr  
 325 330 335  
 Gly Glu Met Leu Asn Thr Leu Ile His His Cys Glu Ala Val Leu His  
 340 345 350  
 Leu Arg Phe Asn Asn Gly Met Met Val Thr Cys Ser Lys Asp Arg Ser  
 355 360 365  
 Ile Ala Val Trp Asp Met Ala Ser Pro Thr Asp Ile Thr Leu Arg Arg  
 370 375 380  
 Val Leu Val Gly His Arg Ala Ala Val Asn Val Val Asp Phe Asp Asp  
 385 390 395 400  
 Lys Tyr Ile Val Ser Ala Ser Gly Asp Arg Thr Ile Lys Val Trp Asn  
 405 410 415  
 Thr Ser Thr Cys Glu Phe Val Arg Thr Leu Asn Gly His Lys Arg Gly  
 420 425 430  
 Ile Ala Cys Leu Gln Tyr Arg Asp Arg Leu Val Val Ser Gly Ser Ser  
 435 440 445  
 Asp Asn Thr Ile Arg Leu Trp Asp Ile Glu Cys Gly Ala Cys Leu Arg  
 450 455 460  
 Val Leu Glu Gly His Glu Glu Leu Val Arg Cys Ile Arg Phe Asp Asn  
 465 470 475 480  
 Lys Arg Ile Val Ser Gly Ala Tyr Asp Gly Lys Ile Lys Val Trp Asp  
 485 490 495  
 Leu Val Ala Ala Leu Asp Pro Arg Ala Pro Ala Gly Thr Leu Cys Leu  
 500 505 510  
 Arg Thr Leu Val Glu His Ser Gly Arg Val Phe Arg Leu Gln Phe Asp

515

520

525

Glu Phe Gln Ile Val Ser Ser Ser His Asp Asp Thr Ile Leu Ile Trp  
 530 535 540

Asp Phe Leu Asn Asp Pro Ala Ala Gln Ala Glu Pro Pro Arg Ser Pro  
 545 550 555 560

Ser Arg Thr Tyr Thr Tyr Ile Ser Arg  
 565

<210> 44  
 <211> 338  
 <212> DNA  
 <213> Mus musculus

<400> 44  
 aagccactga agatggagat ccattttacct agtgtgccga tgatggaaat cctctcctat 60  
 ctggatgcct acagtttgcct acaggctgcc caagtgaaca agaactggaa tgaacttgca 120  
 agcagtgatg tcctgtggag gaagttgtgt cagaagagat ggctctactg ttacatgttc 180  
 accctaccgc tccatggcct agagacatgg aagcagttct tctttaacaa aacatggcaa 240  
 gaacacgcca agaccggggc aaagccagaa gatttcactt acaaggaatt tcctatggag 300  
 tttgaatttc gggcacatcc atggtatatc tcaaggca 338

<210> 45  
 <211> 108  
 <212> PRT  
 <213> Mus musculus

<400> 45  
 Met Glu Ile His Leu Pro Ser Val Pro Met Met Glu Ile Leu Ser Tyr  
 1 5 10 15  
 Leu Asp Ala Tyr Ser Leu Leu Gln Ala Ala Gln Val Asn Lys Asn Trp  
 20 25 30  
 Asn Glu Leu Ala Ser Ser Asp Val Leu Trp Arg Lys Leu Cys Gln Lys  
 35 40 45  
 Arg Trp Leu Tyr Cys Tyr Met Phe Thr Leu Pro Leu His Gly Leu Glu  
 50 55 60  
 Thr Trp Lys Gln Phe Phe Phe Asn Lys Thr Trp Gln Glu His Ala Lys  
 65 70 75 80  
 Thr Arg Ala Lys Pro Glu Asp Phe Thr Tyr Lys Glu Phe Pro Met Glu  
 85 90 95  
 Phe Glu Phe Arg Ala His Pro Trp Tyr Ile Ser Arg  
 100 105

<210> 46  
 <211> 849  
 <212> DNA  
 <213> Homo sapiens

50

```

<400> 46
gcggccgcgc cgcacccgc accggcaccc acgcccacgc ccgaggaagg gcccgcgcgc 60
ggctggggag accgcattcc cttggaaatc ctggtgcaga ttttcgggtt gttggtggcg 120
gcggacggcc ccatgccctt cctgggcagg gctgcgcgcg tgtgccgccg ctggcaggag 180
gccgcttccc aaccgcgcgt ctggcacacc gtgaccctgt cgtccccgct ggtcggccgg 240
cctgccaagg gcgggggtcaa ggccggagaag aagctccttg cttccctgga gtggcttatg 300
cccaatcggg tttcacagct ccagaggctg accctcatcc actggaagtc tcaggtagac 360
cccgtgttga agctggtagg tgagtgtgtg cctcgggtca ctttctcaa gctctccggc 420
tgccacgggt tgactgtgta cgctctggtc atgctagcca aagcctgctg ccagctccat 480
agcctggacc tacagcactc catggtggag tccacagctg tggtagagctt cttggaggag 540
gcagggtccc gaatgcgcaa gttgtggctg acctacagct cccagacgac agccatcctg 600
ggcgcatatg tgggcagctg ctgccccag ctccaggctc tggaggtgag caccggcatc 660
aaccgtaata gcattcccc ttagctgcct gtcgaggctc tgcagaaagg ctgccctcag 720
ctccaggtgc tgcggctgtt gaacctgatg tggctgccc aagcctccggg acgaggggtg 780
gctcccgga caggcttccc tagcctagag gagctctgcc tggcgagctc aacctgcaac 840
tttgtgagc

```

```

<210> 47
<211> 283
<212> PRT
<213> Homo sapiens

```

```

<400> 47
Ala Ala Ala Pro Ala Pro Ala Pro Ala Pro Thr Pro Thr Pro Glu Glu
 1             5             10             15

Gly Pro Asp Ala Gly Trp Gly Asp Arg Ile Pro Leu Glu Ile Leu Val
          20             25             30

Gln Ile Phe Gly Leu Leu Val Ala Ala Asp Gly Pro Met Pro Phe Leu
          35             40             45

Gly Arg Ala Ala Arg Val Cys Arg Arg Trp Gln Glu Ala Ala Ser Gln
          50             55             60

Pro Ala Leu Trp His Thr Val Thr Leu Ser Ser Pro Leu Val Gly Arg
          65             70             75             80

Pro Ala Lys Gly Gly Val Lys Ala Glu Lys Lys Leu Leu Ala Ser Leu
          85             90             95

Glu Trp Leu Met Pro Asn Arg Phe Ser Gln Leu Gln Arg Leu Thr Leu
          100            105            110

Ile His Trp Lys Ser Gln Val His Pro Val Leu Lys Leu Val Gly Glu
          115            120            125

Cys Cys Pro Arg Leu Thr Phe Leu Lys Leu Ser Gly Cys His Gly Val
          130            135            140

Thr Ala Asp Ala Leu Val Met Leu Ala Lys Ala Cys Cys Gln Leu His
          145            150            155            160

Ser Leu Asp Leu Gln His Ser Met Val Glu Ser Thr Ala Val Val Ser
          165            170            175

Phe Leu Glu Glu Ala Gly Ser Arg Met Arg Lys Leu Trp Leu Thr Tyr

```

180	185	190
Ser Ser Gln Thr Thr Ala Ile Leu Gly Ala Leu Leu Gly Ser Cys Cys		
195	200	205
Pro Gln Leu Gln Val Leu Glu Val Ser Thr Gly Ile Asn Arg Asn Ser		
210	215	220
Ile Pro Leu Gln Leu Pro Val Glu Ala Leu Gln Lys Gly Cys Pro Gln		
225	230	235
Leu Gln Val Leu Arg Leu Leu Asn Leu Met Trp Leu Pro Lys Pro Pro		
245	250	255
Gly Arg Gly Val Ala Pro Gly Pro Gly Phe Pro Ser Leu Glu Glu Leu		
260	265	270
Cys Leu Ala Ser Ser Thr Cys Asn Phe Val Ser		
275	280	

<210> 48  
 <211> 1320  
 <212> DNA  
 <213> Homo sapiens

<400> 48  
 ggggtggtgtg tgggggaagc cgcccccggc agcaggatga aacgaggagg aagagatagt 60  
 gaccgtaatt catcagaaga aggaactgca gagaaatcca agaaactgag gactacaaat 120  
 gagcattctc agacttgtga ttggggtaat ctccctcagg acattattct ccaagtattt 180  
 aaatatttgc ctcttcttga ccgggctcat gcttcacaag tttgccgcaa ctggaaccag 240  
 gtatttcaca tgccctgactt gtggagatgt tttgaatttg aactgaatca gccagctaca 300  
 tcttatttga aagctaccca tccagagctg atcaaacaga ttattaaaag acattcaaac 360  
 catctacaat atgtcagctt caagggtggac agcagcaagg aatcagctga agcagcttgt 420  
 gatatactat cgcaacttgt gaattgctct ttaaaaacac ttggacttat ttcaactgct 480  
 cgaccaagct ttatggattt accaaagtct cactttatct ctgcactgac agttgtgttc 540  
 gtaaaactcca aatccctgtc ttcgcttaag atagatgata ctccagtaga tgatccatct 600  
 ctcaaagtac tagtggccaa caatagtgat aactcaagc tgttgaaaat gagcagctgt 660  
 cctcatgtct ctccagcagg tatcctttgt gtggctgatc agtgtcacgg cttaagagaa 720  
 ctagccctga actaccactt attgagtgat gagttgttac ttgcattgtc ttctgaaaaa 780  
 catgttcgat tagaacattt gcgcattgat gtagtcagtg agaactctgg acagacacac 840  
 ttccatacta ttcagaagag tagctgggat gctttcatca gacattcacc caaagtgaac 900  
 ttagtgatgt attttttttt atatgaagaa gaatttgacc ccttctttcg ctatgaaata 960  
 cctgccaccc atctgtactt tgggagatca gtaagcaaag atgtgcttgg ccgtgtggga 1020  
 atgacatgcc ctagactggt tgaactagta gtgtgtgcaa atggattacg gccacttgat 1080  
 gaagagttaa ttcgcattgc agaacgttgc aaaaatttgt cagctattgg actaggggaa 1140  
 tgtgaagtct catgtagtgc ctttgttgag tttgtgaaga tgtgtggtgg ccgcctatct 1200  
 caattatcca ttatggaaga agtactaatt cctgaccaa agtatagttt ggagcagatt 1260  
 cactgggaag tgtccaagca tcttggtagg gtgtggtttc ccgacatgat gccacttgg 1320

<210> 49  
 <211> 428  
 <212> PRT  
 <213> Homo sapiens

<400> 49  
 Met Lys Arg Gly Gly Arg Asp Ser Asp Arg Asn Ser Ser Glu Glu Gly

52

1	5	10	15
Thr Ala Glu Lys Ser Lys Lys Leu Arg Thr Thr Asn Glu His Ser Gln	20	25	30
Thr Cys Asp Trp Gly Asn Leu Leu Gln Asp Ile Ile Leu Gln Val Phe	35	40	45
Lys Tyr Leu Pro Leu Leu Asp Arg Ala His Ala Ser Gln Val Cys Arg	50	55	60
Asn Trp Asn Gln Val Phe His Met Pro Asp Leu Trp Arg Cys Phe Glu	65	70	75
Phe Glu Leu Asn Gln Pro Ala Thr Ser Tyr Leu Lys Ala Thr His Pro	85	90	95
Glu Leu Ile Lys Gln Ile Ile Lys Arg His Ser Asn His Leu Gln Tyr	100	105	110
Val Ser Phe Lys Val Asp Ser Ser Lys Glu Ser Ala Glu Ala Ala Cys	115	120	125
Asp Ile Leu Ser Gln Leu Val Asn Cys Ser Leu Lys Thr Leu Gly Leu	130	135	140
Ile Ser Thr Ala Arg Pro Ser Phe Met Asp Leu Pro Lys Ser His Phe	145	150	155
Ile Ser Ala Leu Thr Val Val Phe Val Asn Ser Lys Ser Leu Ser Ser	165	170	175
Leu Lys Ile Asp Asp Thr Pro Val Asp Asp Pro Ser Leu Lys Val Leu	180	185	190
Val Ala Asn Asn Ser Asp Thr Leu Lys Leu Leu Lys Met Ser Ser Cys	195	200	205
Pro His Val Ser Pro Ala Gly Ile Leu Cys Val Ala Asp Gln Cys His	210	215	220
Gly Leu Arg Glu Leu Ala Leu Asn Tyr His Leu Leu Ser Asp Glu Leu	225	230	235
Leu Leu Ala Leu Ser Ser Glu Lys His Val Arg Leu Glu His Leu Arg	245	250	255
Ile Asp Val Val Ser Glu Asn Pro Gly Gln Thr His Phe His Thr Ile	260	265	270
Gln Lys Ser Ser Trp Asp Ala Phe Ile Arg His Ser Pro Lys Val Asn	275	280	285
Leu Val Met Tyr Phe Phe Leu Tyr Glu Glu Glu Phe Asp Pro Phe Phe	290	295	300
Arg Tyr Glu Ile Pro Ala Thr His Leu Tyr Phe Gly Arg Ser Val Ser	305	310	315
			320



Lys Asp Val Leu Gly Arg Val Gly Met Thr Cys Pro Arg Leu Val Glu  
 325 330 335

Leu Val Val Cys Ala Asn Gly Leu Arg Pro Leu Asp Glu Glu Leu Ile  
 340 345 350

Arg Ile Ala Glu Arg Cys Lys Asn Leu Ser Ala Ile Gly Leu Gly Glu  
 355 360 365

Cys Glu Val Ser Cys Ser Ala Phe Val Glu Phe Val Lys Met Cys Gly  
 370 375 380

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<212> DNA

<213> Homo sapiens

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<400> 51

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Gln Phe Tyr Arg Tyr Tyr Gln Val Ala Arg Asp Val Pro Arg His Pro  
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<400> 54

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&lt;210&gt; 57

&lt;211&gt; 483

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 57

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Ala Leu Ile Cys Pro Pro Asn Leu Pro Gly Phe Gln Asn Gly Arg Gly
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Ser Ser Thr Ser Ser Ser Ser Ile Thr Gly Glu Thr Val Ala Met Val
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His Ser Pro Pro Pro Thr Arg Leu Thr His Pro Leu Ile Arg Leu Ala
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Ser Arg Pro Gln Lys Glu Gln Ala Ser Ile Asp Arg Leu Pro Asp His
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Cys Ala Arg Val Cys Arg Arg Trp Tyr Asn Leu Ala Trp Asp Pro Arg
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Ala Leu Lys Val Leu Thr Arg Arg Leu Cys Gln Asp Thr Pro Asn Val
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 305 310 315 320  
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 370 375 380  
 Gly Val Glu Tyr Leu Ala Lys Asn Cys Thr Lys Leu Lys Ser Leu Asp  
 385 390 395 400  
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 accccgaaga ccacctctcc caccctggga ctgtgtgtcg gggcccgggc tggggattta 300  
 gagccaggcg atccggggcc tatctcggca tcacgctta ccagctcctc ttcgacctca 360  
 gggcctcaat ttctcatcc ctgcaatggg gataaaggac agtacctgcc tcacgggggt 420  
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 atcattcgat aaatgctaatt ttatgactat gttatc 516

<210> 59  
 <211> 160  
 <212> PRT  
 <213> Homo sapiens

<400> 59  
 Met Ala Thr Leu Val Glu Leu Pro Asp Ser Val Leu Leu Glu Ile Phe  
 1 5 10 15  
 Ser Tyr Leu Pro Val Arg Asp Arg Ile Arg Ile Ser Arg Val Cys His  
 20 25 30  
 Arg Trp Lys Arg Leu Val Asp Asp Arg Trp Leu Trp Arg His Val Asp  
 35 40 45  
 Leu Thr Leu Tyr Thr Val Arg Ala Ala Gly Arg Ala Gly Leu Gly Arg  
 50 55 60  
 Gly Arg Gly Ala Arg Thr Pro Lys Thr Thr Ser Pro Thr Leu Gly Leu  
 65 70 75 80  
 Cys Val Gly Ala Arg Ala Gly Asp Leu Glu Pro Gly Asp Pro Gly Pro  
 85 90 95  
 Ile Ser Ala Ser Ser Leu Thr Ser Ser Ser Ser Thr Ser Gly Pro Gln  
 100 105 110  
 Phe Pro His Pro Cys Asn Gly Asp Lys Gly Gln Tyr Leu Pro His Gly  
 115 120 125  
 Ala Ile Met Gly Leu His Glx Asp His Ala Gly Lys Gly Leu Arg Thr  
 130 135 140  
 Val Trp His Ile Phe Ile Ile Arg Glx Met Leu Ile Tyr Asp Tyr Val  
 145 150 155 160

<210> 60

62

<211> 1590  
 <212> DNA  
 <213> Homo sapiens

<400> 60  
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 ggggctgagg cgggagcgag gacacgcccc agagaggaag cagagggagg cggaaagcgtg 180  
 gaggaagggg cgagaggcat catcaaagga gatgagggga gcgtagggggc cgggaaagag 240  
 gcacaaggaa gaaagtatgg gaaggaggaa tggagggtca gggctaggcg gcgggagggc 300  
 gccaggccgg gaagagtaca aggacaagga ggtcagggtt gggcctacat cccggggaca 360  
 ggggcggcca tggcggcgcc agccaggag gaggaggagg aggcggctcg ggagtcagcc 420  
 gcctgcccgg ctgcccggcc agcgctctgg cgccctgccg aagtgtctgt gctgcacatg 480  
 tgctcctacc tcgacatgag ggcctcggc cgccctggcc aggtgtaccg ctggctgtgg 540  
 cacttcacca actgcgacct gctccggcgc cagatagcct gggcctcgct caactccggc 600  
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 atgcagctag aggatgatgc tttgtacata tcccaggcta atttcacctt ggccctaccg 780  
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 gaggacgttt gccactttgt gctggccacc tcgcatattg tcagtgcagg aggagatggg 900  
 aagattggcc ttggtaagat tcacagcacc ttcgctgcca agtactgggc tcatgaacag 960  
 gaggtgaact gtgtggattg caaagggggc atcatatcat ttggctccag ggacaggacg 1020  
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 gaagaccaa tctgggtctgt tgctatcagg ccattactca gctcttttgt gacagggacg 1140  
 gcttgttgtg ggcacttctc acccctgaaa atctgggacc tcaacagtgg gcagctgatg 1200  
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 tgggaccggc accaaagggc ctgcccgcac accttcccgc tgacgtcgac ccgcctcggc 1500  
 agccctgtgt actgcctgca tctcaccacc aagcatctct atgctgcgct gtcttacaac 1560  
 ctccacgtcc tggatattca aaaccctga 1590

<210> 61  
 <211> 529  
 <212> PRT  
 <213> Homo sapiens

<400> 61  
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 20 25 30  
 Asp Gly Glu Gly Gly Ser Gly Pro Gly Ala Glu Ala Gly Ala Arg Thr  
 35 40 45  
 Arg Pro Arg Glu Glu Ala Glu Gly Gly Gly Ser Val Glu Glu Gly Ala  
 50 55 60  
 Arg Gly Ile Ile Lys Gly Asp Glu Gly Ser Val Gly Ala Gly Lys Glu  
 65 70 75 80  
 Ala Gln Gly Arg Lys Tyr Gly Lys Glu Glu Trp Arg Val Arg Ala Arg  
 85 90 95

Arg Arg Glu Gly Ala Arg Pro Gly Arg Val Gln Gly Gln Gly Gly Gln  
 100 105 110  
 Val Trp Ala Tyr Ile Pro Gly Thr Gly Ala Ala Met Ala Ala Ala Ala  
 115 120 125  
 Arg Glu Glu Glu Glu Glu Ala Ala Arg Glu Ser Ala Ala Cys Pro Ala  
 130 135 140  
 Ala Gly Pro Ala Leu Trp Arg Leu Pro Glu Val Leu Leu Leu His Met  
 145 150 155 160  
 Cys Ser Tyr Leu Asp Met Arg Ala Leu Gly Arg Leu Ala Gln Val Tyr  
 165 170 175  
 Arg Trp Leu Trp His Phe Thr Asn Cys Asp Leu Leu Arg Arg Gln Ile  
 180 185 190  
 Ala Trp Ala Ser Leu Asn Ser Gly Phe Thr Arg Leu Gly Thr Asn Leu  
 195 200 205  
 Met Thr Ser Val Pro Val Lys Val Ser Gln Asn Trp Ile Val Gly Cys  
 210 215 220  
 Cys Arg Glu Gly Ile Leu Leu Lys Trp Arg Cys Ser Gln Met Pro Trp  
 225 230 235 240  
 Met Gln Leu Glu Asp Asp Ala Leu Tyr Ile Ser Gln Ala Asn Phe Ile  
 245 250 255  
 Leu Ala Tyr Gln Phe Arg Pro Asp Gly Ala Ser Leu Asn Arg Gln Pro  
 260 265 270  
 Leu Gly Val Ser Ala Gly His Asp Glu Asp Val Cys His Phe Val Leu  
 275 280 285  
 Ala Thr Ser His Ile Val Ser Ala Gly Gly Asp Gly Lys Ile Gly Leu  
 290 295 300  
 Gly Lys Ile His Ser Thr Phe Ala Ala Lys Tyr Trp Ala His Glu Gln  
 305 310 315 320  
 Glu Val Asn Cys Val Asp Cys Lys Gly Gly Ile Ile Ser Phe Gly Ser  
 325 330 335  
 Arg Asp Arg Thr Ala Lys Val Trp Pro Leu Ala Ser Gly Gln Leu Gly  
 340 345 350  
 Gln Cys Leu Tyr Thr Ile Gln Thr Glu Asp Gln Ile Trp Ser Val Ala  
 355 360 365  
 Ile Arg Pro Leu Leu Ser Ser Phe Val Thr Gly Thr Ala Cys Cys Gly  
 370 375 380  
 His Phe Ser Pro Leu Lys Ile Trp Asp Leu Asn Ser Gly Gln Leu Met  
 385 390 395 400  
 Thr His Leu Asp Arg Asp Phe Pro Pro Arg Ala Gly Val Leu Asp Val

405 410 415  
 Ile Tyr Glu Ser Pro Phe Ala Leu Leu Ser Cys Gly Tyr Asp Thr Tyr  
 420 425 430  
 Val Arg Tyr Trp Asp Cys Arg Thr Ser Val Arg Lys Cys Val Met Glu  
 435 440 445  
 Trp Glu Glu Pro His Asn Ser Thr Leu Tyr Cys Leu Gln Thr Asp Gly  
 450 455 460  
 Asn His Leu Leu Ala Thr Gly Ser Ser Phe Tyr Ser Val Val Arg Leu  
 465 470 475 480  
 Trp Asp Arg His Gln Arg Ala Cys Pro His Thr Phe Pro Leu Thr Ser  
 485 490 495  
 Thr Arg Leu Gly Ser Pro Val Tyr Cys Leu His Leu Thr Thr Lys His  
 500 505 510  
 Leu Tyr Ala Ala Leu Ser Tyr Asn Leu His Val Leu Asp Ile Gln Asn  
 515 520 525

Pro

<210> 62  
 <211> 1680  
 <212> DNA  
 <213> Homo sapiens

<400> 62  
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 aaggaggacg gcggcgcgga gttctcggct cgctccagga agaggaaggc aaacgtgacc 120  
 gtttttttgc aggatccaga tgaagaaatg gccaaaatcg acaggacggc gagggaccag 180  
 tgtgggagcc agccttggga caataatgca gtctgtgcag acccctgctc cctgatcccc 240  
 acacctgaca aagaagatga tgaccgggtt taccctaaact caacgtgcaa gcctcggatt 300  
 attgcaccat ccagaggctc cccgctgcct gtactgagct gggcaaatag agaggaagtc 360  
 tggaaaaatca tggttaaaca ggaaaagaca tacttaaggg atcagcactt tcttgagcaa 420  
 caccctcttc tgcagccaaa aatgcgagca attcttcttg attggttaat ggaggtgtgt 480  
 gaagtctata aacttcacag ggagaccttt tacttggcac aagatttctt tgaccgggat 540  
 atggcgacac aagaaaatgt tgtaaaaact cttttacagc ttattgggat ttcattctta 600  
 tttattgcag ccaaacttga ggaaatctat cctccaaagt tgcaccagtt tgcgtatgtg 660  
 acagatggag cttgttcagg agatgaaatt ctcaccatgg aattaatgat tatgaaggcc 720  
 cttaagtggc gtttaagtcc cctgactatt gtgtcctggc tgaatgtata catgcagggt 780  
 gcatatctaa atgacttaca tgaagtgcta ctgccgcagt atcccagca aatctttata 840  
 cagattgcag agctgttggg tctctgtgtc ctggatgttg actgccttga atttccttat 900  
 ggtatacttg ctgcttcggc cttgtatcat ttctcgtcat ctgaattgat gcaaaagggt 960  
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 gttataaggg agacggggag ctcaaaaactg aagcacttca ggggcgtcgc tgatgaagat 1080  
 gcacacaaca tacagacca cagagacagc ttggatttgc tggacaaagc ccgagcaaag 1140  
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 ccacagagcg gtaagaagca gagcagcggg ccggaaatgg cgtgaccacc ccatccttct 1260  
 ccaccaaaga cagttgcgcg cctgctccac gttctcttct gtctgttgca gcggaggcgt 1320  
 gcgtttgctt ttacagatat ctgaatggaa gagtgtttct tccacaacag aagtatttct 1380  
 gtggatggca tcaaacaggg caaagtgttt tttattgaat gcttataggt tttttttaa 1440  
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b5

ggaaggtgct acttgacctt aaggactccc acaacaacaa aagcttgaag ctgtggaggg 1560  
 ccacgggtggc gtggctctcc tcgcaggtgt tctgggctcc gttgtaccaa gtggagcagg 1620  
 tggttgcggg caagcggtgt gcagagccca tagccagctg ggcagggggc tgccctctcc 1680

<210> 63

<211> 395

<212> PRT

<213> Homo sapiens

<400> 63

Met Lys Glu Asp Gly Gly Ala Glu Phe Ser Ala Arg Ser Arg Lys Arg  
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Lys Ala Asn Val Thr Val Phe Leu Gln Asp Pro Asp Glu Glu Met Ala  
 20 25 30

Lys Ile Asp Arg Thr Ala Arg Asp Gln Cys Gly Ser Gln Pro Trp Asp  
 35 40 45

Asn Asn Ala Val Cys Ala Asp Pro Cys Ser Leu Ile Pro Thr Pro Asp  
 50 55 60

Lys Glu Asp Asp Asp Arg Val Tyr Pro Asn Ser Thr Cys Lys Pro Arg  
 65 70 75 80

Ile Ile Ala Pro Ser Arg Gly Ser Pro Leu Pro Val Leu Ser Trp Ala  
 85 90 95

Asn Arg Glu Glu Val Trp Lys Ile Met Leu Asn Lys Glu Lys Thr Tyr  
 100 105 110

Leu Arg Asp Gln His Phe Leu Glu Gln His Pro Leu Leu Gln Pro Lys  
 115 120 125

Met Arg Ala Ile Leu Leu Asp Trp Leu Met Glu Val Cys Glu Val Tyr  
 130 135 140

Lys Leu His Arg Glu Thr Phe Tyr Leu Ala Gln Asp Phe Phe Asp Arg  
 145 150 155 160

Tyr Met Ala Thr Gln Glu Asn Val Val Lys Thr Leu Leu Gln Leu Ile  
 165 170 175

Gly Ile Ser Ser Leu Phe Ile Ala Ala Lys Leu Glu Glu Ile Tyr Pro  
 180 185 190

Pro Lys Leu His Gln Phe Ala Tyr Val Thr Asp Gly Ala Cys Ser Gly  
 195 200 205

Asp Glu Ile Leu Thr Met Glu Leu Met Ile Met Lys Ala Leu Lys Trp  
 210 215 220

Arg Leu Ser Pro Leu Thr Ile Val Ser Trp Leu Asn Val Tyr Met Gln  
 225 230 235 240

Val Ala Tyr Leu Asn Asp Leu His Glu Val Leu Leu Pro Gln Tyr Pro  
 245 250 255

b6

Gln Gln Ile Phe Ile Gln Ile Ala Glu Leu Leu Asp Leu Cys Val Leu  
 260 265 270

Asp Val Asp Cys Leu Glu Phe Pro Tyr Gly Ile Leu Ala Ala Ser Ala  
 275 280 285

Leu Tyr His Phe Ser Ser Ser Glu Leu Met Gln Lys Val Ser Gly Tyr  
 290 295 300

Gln Trp Cys Asp Ile Glu Asn Cys Val Lys Trp Met Val Pro Phe Ala  
 305 310 315 320

Met Val Ile Arg Glu Thr Gly Ser Ser Lys Leu Lys His Phe Arg Gly  
 325 330 335

Val Ala Asp Glu Asp Ala His Asn Ile Gln Thr His Arg Asp Ser Leu  
 340 345 350

Asp Leu Leu Asp Lys Ala Arg Ala Lys Lys Ala Met Leu Ser Glu Gln  
 355 360 365

Asn Arg Ala Ser Pro Leu Pro Ser Gly Leu Leu Thr Pro Pro Gln Ser  
 370 375 380

Gly Lys Lys Gln Ser Ser Gly Pro Glu Met Ala  
 385 390 395

<210> 64  
 <211> 597  
 <212> DNA  
 <213> Homo sapiens

<400> 64  
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 gcgagcacc ccaagccctc ggccctgcagg aacctcttcg gcccgggtgga ccacgaagag 120  
 ttaacccggg acttgagaa gcaactgcaga gacatggaag aggcgagcca gcgcaagtgg 180  
 aatttcgatt ttcagaatca caaaccctta gagggcaagt acgagtggca agaggtggag 240  
 aagggcagct tgcccagatt ctactacaga ccccgcgcc ccccaaaagg tgccctgcaag 300  
 gtgcccggcg aggagagcca ggatgtcagc gggagccgcc cggcgccgcc tttaattggg 360  
 gctccggcta actctgagga cagcatttg gtggacccaa agactgatcc gtcggacagc 420  
 cagacggggt tagcggagca atgcgcagga ataaggaagc gacctgcaac cgacgattct 480  
 tctactcaaa acaaaagagc caacagaaca gaagaaaatg tttcagacgg tcccccaaat 540  
 gccggttctg tggagcagac gcccaagaag cctggcctca gaagacgtca aacgtaa 597

<210> 65  
 <211> 198  
 <212> PRT  
 <213> Homo sapiens

<400> 65  
 Met Ser Asn Val Arg Val Ser Asn Gly Ser Pro Ser Leu Glu Arg Met  
 1 5 10 15

Asp Ala Arg Gln Ala Glu His Pro Lys Pro Ser Ala Cys Arg Asn Leu  
 20 25 30

67

Phe Gly Pro Val Asp His Glu Glu Leu Thr Arg Asp Leu Glu Lys His  
 35 40 45  
 Cys Arg Asp Met Glu Glu Ala Ser Gln Arg Lys Trp Asn Phe Asp Phe  
 50 55 60  
 Gln Asn His Lys Pro Leu Glu Gly Lys Tyr Glu Trp Gln Glu Val Glu  
 65 70 75 80  
 Lys Gly Ser Leu Pro Glu Phe Tyr Tyr Arg Pro Pro Arg Pro Pro Lys  
 85 90 95  
 Gly Ala Cys Lys Val Pro Ala Gln Glu Ser Gln Asp Val Ser Gly Ser  
 100 105 110  
 Arg Pro Ala Ala Pro Leu Ile Gly Ala Pro Ala Asn Ser Glu Asp Thr  
 115 120 125  
 His Leu Val Asp Pro Lys Thr Asp Pro Ser Asp Ser Gln Thr Gly Leu  
 130 135 140  
 Ala Glu Gln Cys Ala Gly Ile Arg Lys Arg Pro Ala Thr Asp Asp Ser  
 145 150 155 160  
 Ser Thr Gln Asn Lys Arg Ala Asn Arg Thr Glu Glu Asn Val Ser Asp  
 165 170 175  
 Gly Ser Pro Asn Ala Gly Ser Val Glu Gln Thr Pro Lys Lys Pro Gly  
 180 185 190  
 Leu Arg Arg Arg Gln Thr  
 195

<210> 66  
 <211> 1600  
 <212> DNA  
 <213> Homo sapiens

<400> 66  
 gaattccggg ctgtagagct tgcccgcgca gtgggggatgg aacgttgcta ggcttagcgg 60  
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 ggaaggcgaa gcagctctgc aagtttaatg cacgtattta aaactcccg gcctgcggac 180  
 gctatgcaca ggaagcacct ccaggagatt ccagacctga gtagcaacgt tgccaccagc 240  
 ttcacgtggg gatgggatcc cagcaagact tctgaactgc tgtcaggcat ggggggtctcc 300  
 gccctggaga aagaggagcc cgacagttag aacatcccc aggaactgct ctcaaacctg 360  
 ggccacccgg agagccccc acggaaacgg ctgaagagca aaggagtgta caaagacttt 420  
 gtaattgtcc gcaggcctaa gctaaatcgg gagaactttc caggtgtttc atgggactct 480  
 cttccggatg agctgctctt gggaatcttt tctgtctgt gcctccctga gctgctaaag 540  
 gtctctggtg tttgtaagag gtggtatcgc ctacgctctg atgagctct atggcagacc 600  
 ttagacctta caggtaaaaa tctgcaccg gatgtgactg gtcggttgct gtctcaaggg 660  
 gtgattgcct tccgctgcc acgatcattt atggaccaac cattggctga acatttcagc 720  
 ccttttcgtg tacaggacat ggacctatcg aactcagtta tagaagtgtc caccctccac 780  
 ggcatactgt ctacgtgttc caagttgcag aatctaagcc tggaactgcg gctttcggat 840  
 cccattgtca atactctcgc aaaaaactca aatttagtgc gacttaacct tctgggtgtg 900  
 cctggattcc ctaaatttcc cctgcagact ttcctaagca gctgtcccag actggatgag 960  
 ctgaacctct cctggtgttt taatttcact gaaaagcatg tacaggtggc tgttgccat 1020  
 gtctcagaga ccatgaccca gctgaatcta agcggctaca gaaagaatct ccagaaatca 1080

68

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gatctctcta ctttagttag aagatgcccc aatcttgtcc atctagactt aagtaatatg 1140
gtcatgctaa agaatgactg ctttcaggaa ttttccagc tcaactacct ccaacaccta 1200
tcactcagtc ggtgctatga tataatacct gaaactttac ttgaacttgg agaaattccc 1260
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gaagcccttc ctcacttaca gattaattgc tcccatttca ccaccattgc caggccaact 1380
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cccagttgtc tatgaagtat ttattgcagg atggtgtctc ttcttttagaa cagggaaaat 1500
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ctgtcattct gcaagtatac tagggagccc attttgagag 1600

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<210> 67  
 <211> 435  
 <212> PRT  
 <213> Homo sapiens

<400> 67  
 Met His Val Phe Lys Thr Pro Gly Pro Ala Asp Ala Met His Arg Lys  
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 His Leu Gln Glu Ile Pro Asp Leu Ser Ser Asn Val Ala Thr Ser Phe  
 20 25 30  
 Thr Trp Gly Trp Asp Ser Ser Lys Thr Ser Glu Leu Leu Ser Gly Met  
 35 40 45  
 Gly Val Ser Ala Leu Glu Lys Glu Glu Pro Asp Ser Glu Asn Ile Pro  
 50 55 60  
 Gln Glu Leu Leu Ser Asn Leu Gly His Pro Glu Ser Pro Pro Arg Lys  
 65 70 75 80  
 Arg Leu Lys Ser Lys Gly Ser Asp Lys Asp Phe Val Ile Val Arg Arg  
 85 90 95  
 Pro Lys Leu Asn Arg Glu Asn Phe Pro Gly Val Ser Trp Asp Ser Leu  
 100 105 110  
 Pro Asp Glu Leu Leu Leu Gly Ile Phe Ser Cys Leu Cys Leu Pro Glu  
 115 120 125  
 Leu Leu Lys Val Ser Gly Val Cys Lys Arg Trp Tyr Arg Leu Ala Ser  
 130 135 140  
 Asp Glu Ser Leu Trp Gln Thr Leu Asp Leu Thr Gly Lys Asn Leu His  
 145 150 155 160  
 Pro Asp Val Thr Gly Arg Leu Leu Ser Gln Gly Val Ile Ala Phe Arg  
 165 170 175  
 Cys Pro Arg Ser Phe Met Asp Gln Pro Leu Ala Glu His Phe Ser Pro  
 180 185 190  
 Phe Arg Val Gln Asp Met Asp Leu Ser Asn Ser Val Ile Glu Val Ser  
 195 200 205  
 Thr Leu His Gly Ile Leu Ser Gln Cys Ser Lys Leu Gln Asn Leu Ser  
 210 215 220



Leu Glu Leu Arg Leu Ser Asp Pro Ile Val Asn Thr Leu Ala Lys Asn  
 225 230 235 240  
 Ser Asn Leu Val Arg Leu Asn Leu Pro Gly Cys Pro Gly Phe Pro Lys  
 245 250 255  
 Phe Pro Leu Gln Thr Phe Leu Ser Ser Cys Pro Arg Leu Asp Glu Leu  
 260 265 270  
 Asn Leu Ser Trp Cys Phe Asn Phe Thr Glu Lys His Val Gln Val Ala  
 275 280 285  
 Val Ala His Val Ser Glu Thr Met Thr Gln Leu Asn Leu Ser Gly Tyr  
 290 295 300  
 Arg Lys Asn Leu Gln Lys Ser Asp Leu Ser Thr Leu Val Arg Arg Cys  
 305 310 315 320  
 Pro Asn Leu Val His Leu Asp Leu Ser Asn Ser Val Met Leu Lys Asn  
 325 330 335  
 Asp Cys Phe Gln Glu Phe Ser Gln Leu Asn Tyr Leu Gln His Leu Ser  
 340 345 350  
 Leu Ser Arg Cys Tyr Asp Ile Ile Pro Glu Thr Leu Leu Glu Leu Gly  
 355 360 365  
 Glu Ile Pro Thr Leu Lys Thr Leu Gln Val Phe Gly Ile Val Pro Asp  
 370 375 380  
 Gly Thr Leu Gln Leu Leu Lys Glu Ala Leu Pro His Leu Gln Ile Asn  
 385 390 395 400  
 Cys Ser His Phe Thr Thr Ile Ala Arg Pro Thr Ile Gly Asn Lys Lys  
 405 410 415  
 Asn Gln Glu Ile Trp Gly Ile Lys Cys Arg Leu Thr Leu Gln Lys Pro  
 420 425 430  
 Ser Cys Leu  
 435

&lt;210&gt; 68

&lt;211&gt; 1455

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 68

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 aagttgcaga gttctgatgg agagatattt gaagttgatg tggaaattgc caaacaattct 180  
 gtgactatta agaccatggt ggaagatttg ggaatggatg atgaaggaga tgatgaccca 240  
 gttcctctac caaatgtgaa tgcagcaata ttaaaaaagg tcattcagtg gtgcacccac 300  
 cacaaggatg accctcctcc tcctgaagat gatgagaaca aagaaaagcg aacagatgat 360  
 atccctgttt gggaccaaga attcctgaaa gttgaccaag gaacactttt tgaactcatt 420  
 ctggctgcaa actacttaga catcaaaggt ttgcttgatg ttacatgcaa gactgttgcc 480  
 aatatgatca aggggaaaac tcctgaggag attcgcaaga ccttcaatat caaaaatgac 540

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tttactgaag aggaggaagc ccaggtacgc aaagagaacc agtgggtgtga agagaagtga 600
aatgttggtgc ctgacactgt aacactgtaa ggattgttcc aaatactagt tgcactgctc 660
tggtttataat tggttaatat agacaaacag tagacaaatg cagcagcaag tcaattgtat 720
tagcagaata ttgtcctcat tgcattgtga gttgtagctc gagtcccaaa ccttacggcc 780
aagtttcttc tagtatgatg gaaagtttct tttttctttg ctctgaataa aactgaactg 840
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tagtttattg tccaagttaa ctttaggtga ccttttaaaa gttggcattg aaaataaaac 960
aaacttgcaaa aaagttttct ggaatagaat taacaaaata ttatctttat catgagttgg 1020
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gcctcctgca gtcaaggagt accactgtat tgattagcct gtatgtagca gggctccctt 1140
cattgcatct gaggacttgt tttctttttc tttattttta atcctcttag ttttaaata 1200
attgcctaga gactcagtta ctaccagtt tgtgggtttt tgggagaaat gtaactggac 1260
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gtgttcccat gtggagaaaa ttattcacac tacttgcag taaagaataa ttttaacttt 1380
aacattaaaa tatgtggtaa aaccagaaa gcatccatca tgaatgcaag atactttcaa 1440
taaagtaagt tatat 1455

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<210> 69  
 <211> 163  
 <212> PRT  
 <213> Homo sapiens

<400> 69  
 Met Pro Ser Ile Lys Leu Gln Ser Ser Asp Gly Glu Ile Phe Glu Val  
 1 5 10 15  
 Asp Val Glu Ile Ala Lys Gln Ser Val Thr Ile Lys Thr Met Leu Glu  
 20 25 30  
 Asp Leu Gly Met Asp Asp Glu Gly Asp Asp Asp Pro Val Pro Leu Pro  
 35 40 45  
 Asn Val Asn Ala Ala Ile Leu Lys Lys Val Ile Gln Trp Cys Thr His  
 50 55 60  
 His Lys Asp Asp Pro Pro Pro Pro Glu Asp Asp Glu Asn Lys Glu Lys  
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74

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## INTERNATIONAL SEARCH REPORT

Int l Application No

PCT/US 00/15449

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 C07K14/47 A61K38/17 G01N33/68 A61P43/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

MEDLINE, CHEM ABS Data, WPI Data, PAJ, BIOSIS, EPO-Internal, STRAND

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 11176 A (BEACH DAVID ;ZHANG HUI (US); COLD SPRING HARBOR LAB (US)) 27 March 1997 (1997-03-27) the whole document	1,2,6,7, 9,14-20, 27
X	YU Z K ET AL: "Human CUL-1 associates with the SKP1/ SKP2 complex and regulates p21(CIP1/WAF1) and cyclin D proteins." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 SEP 15) 95 (19) 11324-9. , XP002151555 cited in the application See especially page 11328, discussion the whole document	1,2,6,7, 9,14-19

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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\* &amp; \* document member of the same patent family

Date of the actual completion of the international search

8 November 2000

Date of mailing of the international search report

22/11/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Groenendijk, M



## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/15449

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PAGANO E.A.: "Cell cycle regulation by the ubiquitin pathway" FASEB J., vol. 11, 1997, pages 1067-1075, XP002151556 See especially page 1069 and Table 2	1,2,4,5
X	WO 99 18989 A (BAYLOR COLLEGE MEDICINE) 22 April 1999 (1999-04-22) the whole document	1-5
X	IATAKEYAMA E.A.: "Ubiquitin-dependent degradation of I $\kappa$ B $\alpha$ is mediated by a ubiquitin ligase Skp1/Cul-1/F-box protein FWD1" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 96, March 1999 (1999-03), pages 3859-3863, XP002152297 WASHINGTON US the whole document	1,3
X	ZHANG H ET AL: "p19Skp1 and p45Skp2 are essential elements of the cyclin A-CDK2 S phase kinase." CELL, (1995 SEP 22) 82 (6) 915-25. , XP002151557 cited in the application The whole document; see especially page 920, column 2	14-19
X	CHAO E.A.: "Overexpression of cyclin A but not SKP2 correlates with the tumor relapse of human hepatocellular carcinoma" CANCER RESEARCH, vol. 58, 1 March 1998 (1998-03-01), pages 985-990, XP002151558 See especially abstract	14-19
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International Application No

PCT/US 00/15449

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	CARRANO E.A.: "SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27" NATURE CELL BIOLOGY, vol. 1, August 1999 (1999-08), pages 193-199, XP002151559 cited in the application the whole document	1,2, 4-21, 23-28
P,X	SUTTERLÜTY E.A.: "P45(skp2) promotes p27(kip1) degradation and induces S phase in quiescent cells" NATURE CELL BIOLOGY, vol. 1, August 1999 (1999-08), pages 207-214, XP002151560 cited in the application the whole document	1,2, 4-21, 23-28
P,X	CENCIARELLI E.A.: "Identification of a family of human F-box proteins" CURRENT BIOLOGY, vol. 9, 11 October 1999 (1999-10-11), pages 1177-1179, XP000953487 the whole document	1-5
P,X	WINSTON E.A.: "A family of mammalian F-box proteins" CURRENT BIOLOGY, vol. 9, 11 October 1999 (1999-10-11), pages 1180-1192, XP000960309 the whole document	1-5
A	MONTAGNOLI E.A.: "Ubiquitination of p27 is regulated by Cd-dependent phosphorylation and trimeric complex formation" GENES AND DEVELOPMENT, vol. 13, no. 9, 1 May 1999 (1999-05-01), pages 1181-1189, XP002151561 cited in the application the whole document	

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Information on patent family members

International Application No

PCT/US 00/15449

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO 9711176	A	27-03-1997	US 5981702 A CA 2230138 A	09-11-1999 27-03-1997
WO 9918989	A	22-04-1999	AU 1088399 A	03-05-1999

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